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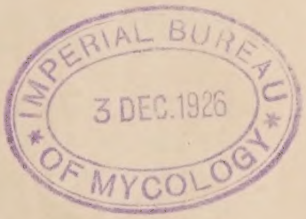
STUDIES ON TREATMENT OF COTTON SEED

BY
S. G. LEHMAN



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AGRICULTURAL EXPERIMENT STATION
OF THE
NORTH CAROLINA STATE COLLEGE OF AGRICULTURE
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SUMMARY

1. Anthracnose is one of the most destructive diseases of cotton in the United States. This disease causes losses amounting to many thousands of dollars annually in North Carolina alone. It is caused by a fungus which, under present cultural practices, is known to be spread chiefly in the form of spores and mycelium lodged upon the surface and within the cotton seed.

2. The methods commonly recommended for the control of seed-borne anthracnose of cotton are selection of seed from healthy plants, seed sterilization with chemical disinfectants, corrosives, and hot water and use of old seed for planting. Each of these methods is encumbered by certain defects which make desirable the development of a more certain and more effective method of control.

3. Other investigators have with more or less success attempted to control seed-borne diseases of other plants by the use of dry heat.

4. This study on the control of cotton anthracnose began with the use of seed of the 1919 crop and a new supply of infected seed was obtained each season. The seeds were heated in quantities of 50 or 100 in an electric oven provided with a constant temperature regulator. They were germinated individually on moist blotting paper in test tubes and each diseased seedling was examined microscopically to determine the nature of the fungus attacking it.

5. Certain preliminary experiments dealing with germination of cotton seeds and growth of the anthracnose fungus show that temperatures of 25 and 30° C. are not only very favorable for germination of cotton seed and the subsequent growth of the seedlings but also closely approximate the optimum for growth in culture of the fungus causing cotton anthracnose and for the development of the disease on seedlings. A temperature of 35° C. permits good germination of cotton seed but inhibits the growth of the fungus which causes anthracnose and prevents the development of the disease. At 20° C. germination of cotton seed and growth of the seedlings are good, but growth of the anthracnose fungus and the development of the disease are slower than at higher temperatures.

6. The germinability of air dry cotton seed was completely destroyed in 15 minutes by dry heat at 90 and 100° C. and seriously impaired after one hour at 80° C. A temperature of 70° C. for 24 hours impaired the germination of seed containing an amount of moisture normal

to ordinary storage conditions, but did not reduce germination of seed whose water content had been reduced to 8.3 per cent by storage under laboratory conditions. Anthracnose was greatly reduced by heating seed at 70° C. for 24 hours but was not completely eliminated by treatment at this temperature for 48 hours.

7. A temperature of 60° C. applied to air dry cotton seed either directly or after a period of preliminary drying at a lower temperature did not kill all the anthracnose.

8. Cotton seed which had been predried at temperatures of 40 to 50° C. for 24 hours or more were subjected to a temperature of 70° C. for 72 hours without marked loss of viability, but this treatment did not free the seed from anthracnose.

9. When infected cotton seed were predried at 40 to 45° C. for 24 to 72 hours or at 50° C. for 12 to 24 hours and heated at 80° for 24 to 72 hours, complete elimination of anthracnose was obtained in every test except one (5 out of 6) in which the heating was continued to 72 hours, but was secured in none of the tests in which heating stopped at the end of 48 hours. Little or no reduction of germination occurred as a result of these treatments.

10. When predried cotton seed were heated at 90° C., all anthracnose was killed in 24 hours in 7 out of 8 tests, in less than 24 hours in 7 out of 13 tests, and in 48 or 72 hours in the 3 remaining tests. No significant reduction in germination resulted from the treatment at 90° C. when the seed had been predried at 50° C. or above for 24 hours.

11. When predried cotton seed were heated at 100° C., complete control of anthracnose was secured in all except one (7 out of 8) test, and in this one instance the seed were heated for only 2 hours. Heating at 100° greatly reduced the germinability of the seed which had been predried at 50° C. or lower for 24 hours. Much better protection was obtained by predrying the seed at 60° C. for 24 hours.

12. When predried seed were heated at 95° C., complete control of anthracnose was secured in all of 14 tests in which the seed were heated longer than 8 hours, and in 4 out of the 6 remaining tests in which the seed were heated for 8 hours or less. Predrying the seed at 50° C. for 36 hours or at 60° for 18 to 24 hours conditioned the seed to endure the temperature of 95° C. without reduction in percentage of germination.

13. A machine for treating cotton seed in bulk with dry heat is described and illustrated. This machine is dependable and partially automatic in operation and requires only occasional attention of the operator. The viability of the seed-borne elements of the anthracnose

fungus present in infected seed may be destroyed by use of this machine. In this machine, the effective treatment for control of the disease without serious diminution of germinability consists of 20 to 24 hours of desiccation at 60-65° C. followed by 12 hours of heating at 95-100° C.

14. The moisture content of cotton seeds is a factor which strongly modifies their resistance to heat and which may be used as a criterion of their ability to withstand the temperatures that are effective in the control of anthracnose. Without exception, when the moisture content after the preliminary drying was as great as 3.9 per cent of the oven dry weight, the viability of the seeds was seriously impaired by heating at 95° for 12 hours. When the moisture content after the preliminary drying was not greater than 3.62 per cent of the dry weight of the seeds, no serious loss of viability resulted from the treatment at 95°.

15. When the water content after drying was not greater than 3.19 per cent of their dry weight, the seeds heated at 95° for 12 hours germinated more promptly than the untreated seeds used for a check. This accelerated germination is not due to any wholesome effect of the treatment on the embryo of the seed, but is to be attributed to changes in the seed coat which facilitate access of water to the embryo. Naked embryos from treated seed germinate somewhat less promptly than treated embryos, but when seeds are germinated with coats intact, this effect is completely masked and amended by the favorable changes in the seed coat.

16. The viability of the seed-borne elements of the anthracnose fungus was prolonged by storing infected seed over such desiccating chemicals as concentrated H_2SO_4 and CaO. This result shows that the death of the anthracnose fungus when infected seeds are treated with dry heat is not due to the desiccation accompanying the treatment but to a more direct effect of the heat on the fungus protoplasm. The necessary preliminary desiccation serves only to prepare the seeds to endure the high temperature used in the final stage of the treatment.

17. The thorough desiccation which occurs when cotton seeds are stored for long periods over concentrated H_2SO_4 and dry CaO, induces a condition of more or less complete secondary dormancy in many of the seeds. This condition is slowly reversible and is characterized by a very hard and dry condition of the seed coat which prevents absorption of water. The embryos of such seed germinate promptly upon removal of the seed coat.

18. The storage of infected cotton seed in hydrogen and carbon dioxide does not free the seeds from anthracnose.

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STUDIES ON TREATMENT OF COTTON SEED

By Samuel G. Lehman

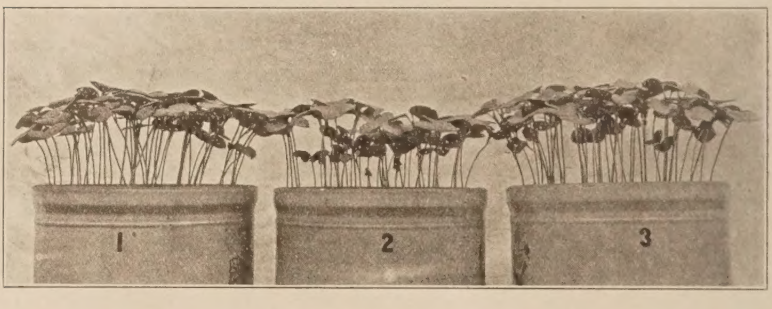
PREFATORY STATEMENT

This investigation has, from its inception in 1920, been concerned primarily with the control of cotton anthracnose, although studies have been made of a number of other seed-borne diseases. Consideration has been given mainly to treatment with dry heat and, as might be anticipated, high temperatures have been found to influence not only the viability of the parasite but also the germination of the cotton seed themselves. The study has included the influence and interrelationship of the several factors, degree of heat, duration of the period of heating and moisture content of the seed, on germination, enzymic activity and respiration of the seed and on the viability of the fungus. Since the work on those portions dealing with the influence of dry heat on enzymic and respiratory activity is not yet completed, it is planned to report on them in the near future as separate series of these "Studies on Treatment of Cotton Seed" and to include in the present papers the completed phases of this work. These data naturally group themselves under two general headings, (1) The control of cotton anthracnose by the use of dry heat and (2) Germination of cotton seed and viability of anthracnose as influenced by desiccation and by storage in inert gases.

ECONOMIC IMPORTANCE OF COTTON ANTHRACNOSE

Anthracnose of cotton is one of the most destructive of all the diseases common to this important plant in the United States. As the result of the presence of this disease, cotton growers, breeders and seedsmen suffer loses amounting to thousands of dollars annually. Destruction of 30 to 50 per cent of the bolls in some fields is not an infrequent occurrence and cases are known where the losses amounted to 80 or 90 per cent of the crop. A fair idea of the amount of loss caused by this disease may be gained from a consideration of estimates published by the Plant Disease Survey.[25] These figures show that in 1923 all cotton diseases combined caused a reduction in yield of 2,439,000 bales in the United States. Nearly one-tenth of this loss, or a reduction of 229,000 bales is attributed to anthracnose alone. In the same year, the loss to North Carolina cotton producers caused by anthracnose amounted to 36,000 bales. These estimates do not include the concomitant reduction in yield of seed nor the losses arising from the destruction of the stand during the seedling stage. As a result of the use of diseased seed, it is often necessary to replant entire fields and in consequence to lose valuable time and labor from other urgent springtime operations.

Cotton anthracnose is caused by the fungus *Glomerella gossypii*. This fungus attacks the cotton plant in all stages of its development. If infected seed have been planted the seedlings will be attacked and diseased areas will appear on cotyledons and stems. If cool, moist weather prevails most of the diseased seedlings will perish. More favorable weather conditions make it possible for many of the diseased seedlings to survive the attack of the fungus and to grow to mature plants. Such plants are, however, not free from the disease, but, on the contrary, bear many diseased bolls and serve as centers from which neighboring plants may become infected.



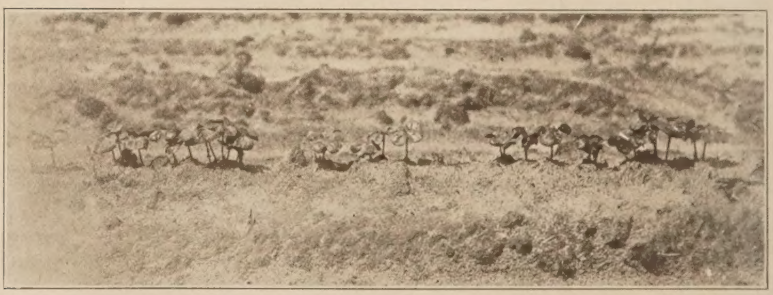


Plate 3. Top. Cotton seeds treated in the machine described in this bulletin and planted in jars in the greenhouse. 50 seeds were planted in each jar and the picture was taken 11 days later. The seed planted in jar 1, had been dried at an average temperature of 51° C. for 24 hours then heated at 90° C. for 22 hours. The seed planted in jar 3 had been dried at 40-45° C. for 24 hours, and subsequently heated at 80° C. for 72 hours. The seedlings in pot 3 came from a comparable lot of untreated seed. Germination was as follows: jar 1, 96 per cent; jar 2, 98 per cent; jar 3, 98 per cent. The treated seed germinated as well and produced somewhat more vigorous seedlings than untreated seed. Bottom. A close-up view of some of the plants shown in Plate 4.

While cotton is growing from the seedling to the fruiting stage the fungus lives and sporulates on the cotyledons, stems, leaves and leaf scars. It has recently been reported[10] that anthracnose grows in the nectar glands of leaves of infected plants. This may serve as another source of infection for bolls which develop later. The greatest and most obvious loss arises from damage to the bolls. The fungus grows within the tissues of the

bolls and produces more or less discoloration or even complete decay of both fiber and seeds. Individual bolls may fail entirely to open or if less severely attacked only a part of the locks may open. In such cases, the lint is frequently so badly discolored or so firmly matted together as to be worthless. Even when infection is so slight as to produce no apparent discoloration, the microscope will reveal the presence of the fungus filaments among the fibres and within the tissues of the seed.

NATURE OF THE SEED-BORNE INFECTION

By the planting of infected seed, it is easily demonstrable that the fungus which causes cotton anthracnose is carried from season to season on the seed. This fact may be established by germinating on sterile blotters seed which came from diseased bolls. Some or all of the seedlings will develop anthracnose lesions on cotyledons or hypocotyl, whereas seed from uninfected bolls will produce healthy seedlings only. A large portion of this inoculum is carried in the form of spores and mycelium in the fuzz on the surface of the seed. If diseased seed which have been delinted with sulphuric acid are germinated it is found that the percentage of diseased seedlings is very much less than that obtained by germinating untreated seed from the same source. However, the most severe treatment with surface disinfectants will not kill all the seed borne elements of this disease. This situation indicates that the fungus causing anthracnose must be carried in part within the seed. Barre[3] has described and clearly illustrated this condition by drawings made from sections of diseased seeds which show that the mycelium penetrates the seed coat and invades the tissues of the embryo often sporulating on its surface. Here it remains in a dormant but viable condition throughout the winter and starts into active growth when the seeds germinate in the following spring. Any seed treatment, therefore, which is directed toward the control of cotton anthracnose must reckon with the the internal nature of the infection in order to attain any satisfactory measure of success.

METHODS COMMONLY RECOMMENDED FOR CONTROL OF ANTHRACNOSE

A. *Seed selection.* Field selection of seed is a practice which cannot be too highly recommended as a matter of general farm procedure. It is advisable from an agronomic as well as a pathological point of view. Very encouraging results have been obtained in the control of cotton anthracnose by taking seed from plants which bear no diseased bolls and which are not adjacent to other diseased plants. By selecting seed from healthy plants in a field where 20 per cent of the bolls were infected Barre[3] obtained a crop free from anthracnose, whereas the crop from unselected seed from the same field was abundantly diseased. However, when the average person, whose eye is not trained to recognize infections of an incipient or minor nature, makes the selections, a small amount of diseased seed is liable to be included unwittingly. Moreover, in cotton breeding operations small plots representing strain or type selections may become diseased as the result of wind or insect borne spores from

neighboring fields. If, in such a case, one is forced to select seed from only a part of the plants because of the presence of disease on the others, the quantity of seed available for the multiplication plots is greatly reduced. Seedsmen and breeders who have had this experience appreciate the desirability of a dependable method of treatment for killing all seed-borne infection without reducing the capacity of the treated seed for germination. Moreover, this would make possible the introduction with safety of seed of desirable lineage whose disease record is unknown.

B. *Surface disinfection.* Treatment of diseased cotton seed with such surface disinfectants as solutions of copper sulphate, mercuric chloride and formaldehyde is difficult because of the fuzz on the surface of the seed. These solutions do not readily enter the interstices of the fuzz; hence a large portion of the surface borne infection escapes their action[4, 19, 33]. Moreover, such disinfectants cannot reach the elements of the fungus carried within the embryonic tissues without seriously damaging the embryo itself.

Delinting with sulphuric acid has also been used as a measure of control of cotton anthracnose. This solution dissolves not only the lint, but also any spores and mycelium that may be carried in it. Such treatment greatly diminishes the load of infective material carried by the seed and decreases the incidence of the disease in the field. However, it does not render infected seed completely free from disease since delinting does not reach mycelium and spores carried within the seed. This fact is clearly demonstrated by tests made by the writer in which highly diseased seeds were delinted with sulphuric acid and compared with nondelinted seeds in order to determine the relative number of seeds in which the fungus had penetrated beyond the reach of this powerful disinfectant. Each test comprised 50 treated and 50 untreated seeds selected to exclude any which appared to be nonviable. The seeds were kept in concentrated sulphuric acid for periods of 10, 20 and 30 minutes. During this time they were repeatedly stirred to insure good contact with this acid. At the end of the periods indicated the seeds were washed in several changes of tap water to remove the acid and finally placed on sterile, moist blotting paper in test tubes to germinate. The result of these tests are presented in Table I.

TABLE I

Percentage of Anthracnose Which Developed on Seedlings From Corresponding Lots of Delinted and Undelinted Cotton Seed.

Lot No.	Minutes in Con. H_2SO_4	Days in Germinator	Per Cent of Seed Which Developed Diseased Seedlings		Per Cent of Germination		Year When Grown
			Treated	Check	Treated	Check	
1	20	8	6	66	64	98	1919
2	10	8	12	34	82	98	1919
3	10	8	10	34	70	98	1919
4	30	14	16	----------	92	----------	1922

In test 1, 6 per cent of the treated as compared with 66 per cent of the untreated seed produced diseased seedlings. Likewise, in tests 2 and 3, a marked reduction in number of diseased seedlings resulted from the treatment. However, in none of the four tests did delinting with sulphuric acid completely suppress the disease. With the 10 minute treatment a small amount of fuzz still persisted at the large end of the seed and this may have prevented the complete destruction of such infective material as may have lodged in it. However, with the 30 minute treatment, the seed coats were much corroded and the lint was so completely removed that it seemed impossible for any spores or surface borne material to survive. Crawford[17] supplemented the usual treatment with sulphuric acid by subjecting delinted, washed and dried seed to a second charring with sulphuric acid and a subsequent treatment with a 1-1000 solution of mercuric chloride. He reports that, when such seed were germinated on sterile blotting paper, cultures of *Glomerella gossypii Diplodia gossypii*, *Cephalothecium* sp., *Alternaria* sp. and *Fusarium vasinfectum* were obtained from them. These tests make obvious the conclusion that while seedling infection may be greatly diminished it cannot be completely eliminated by delinting.

C. *Hot water.* In 1910, Duggar and Cauthen[10] testing the efficiency of various seed treatments found that the amount of seed borne infection in the case of cotton anthracnose could be greatly lessened by immersing diseased seeds for 22 minutes in water of a temperature of 150° F. Of the bolls which grew on these treated plants, 2.4 per cent became diseased compared with 9.9 per cent on plants from untreated seed. The marked reduction in percentage of diseased bolls apparently resulting from this treatment aroused the hope that the complete elimination of seed-borne infection might be accomplished. In 1914, Barre and Aull[12] published a preliminary report of their work with the hot water treatment. They found that seeds could be kept in water at 70° C. for 15 minutes without injury to germination and, when so treated, diseased seed germinated free from disease, while disease appeared on 22 per cent of the seedlings from the untreated seed. However, in field tests of seed which had been treated at 75° C. for 15 minutes made during this and subsequent years,[5, 6, 12] a small amount of disease developed on the plants grown from treated seed. This amounted to much less than one per cent of the bolls on plants from treated seed, whereas 15 per cent of the bolls from untreated seed became diseased.

The method is encumbered by certain difficulties of operation. It appears that a temperature of less than 70° C. for fifteen minutes is too low to destroy the seed-borne infection, while a temperature of more than 75° C. for 15 minutes causes unfavorable reduction in germination of the seed treated[10, 12] Barre[6] has stated that some varieties of cotton are killed at 72° C. while others will tolerate 76° C. These close temperature limits for the killing of the seed and of the fungus make it difficult in practice to maintain the water at a proper temperature when seed are treated in quantity. This operation is troublesome since it involves a suitable place for drying the seed and considerable time and labor. Moreover, after cotton seed have been treated by the hot water method, they must be dried

before they can be stored again. Thus, while the hot water treatment greatly diminishes the number of seeds carrying viable elements of the anthracnose organism and greatly reduces the incidence of the disease in the field, it does not appear that the method can be depended upon to completely eradicate seed-borne infection.

D. *Ageing of seed.* In 1910 Barre[8] planted seed of the 1908 crop on disease-free land and about 10 per cent of the bolls became diseased whereas plants from healthy seed remained free. Seed from the same lot was planted again in 1911, when less than one per cent of the bolls developed disease. Germination tests of this same seed revealed no diseased seedlings. This indicates that seeds carrying the anthracnose fungus in a viable condition are very few in number by the middle of the third year. In 1916[7] he stated that "Three-year-old seed kept in our office and laboratories continued to give disease-free plants when planted in the field," but that "some of the seed claimed by farmers to be three years old, and kept in sheds or barns did not give such satisfactory results. . . ."

Edgerton[20] made successive plantings of infected seed in the greenhouse and found that no diseased plantlets came from seeds which had attained the age of 13 months. Cultures made from seed gave results in harmony with those obtained from the greenhouse experiments. The writer's observations on this point show that seed of the 1919 crop, germinated in test tubes when 9 months old produced seedlings 58 per cent of which were diseased. On April 16, 1921, 18½ months from picking time, 4 per cent of the seeds still bore viable elements of the anthracnose fungus. During the nine months immediately following picking, these seed were stored in an old barn and were for the remaining 9½ months kept in a closed can in the laboratory. In another test, seed of the 1921 crop produced 20 per cent of infected seedlings when 17½ months old and 2 per cent when 21½ months old. In two tests made with seed which were heavily infected when harvested and which were older than 21½ months, no disease appeared on the seedlings. It should be stated, however, that these seed were stored in the laboratory and were not, therefore, subjected to the changes which obtain in gins and barns, places where seed are usually stored. In view of these observations, it seems probable that, for North Carolina conditions, diseased seed may be planted safely in the third season after picking.

Ageing is the only process yet described for completely freeing infected cotton seed from anthracnose. This method has certain objectionable features, however, the most obvious of which are:

1. Storage space must be provided for seed from three seasons' crops.
2. It quadruples the required storage period and proportionally increases the losses arising from fire, rains and rodents.
3. Breeders cannot by this method free their seed from infection without serious loss of time and very great inconvenience.

Consideration has been given in the above paragraphs to the various methods heretofore recommended as helpful in the control of seed-borne infection by anthracnose. None of these measures yield thoroughly satisfactory results under all conditions. There is evident need of a method

of treatment which can be used at will to effectually destroy all seed-borne infection. It is the purpose of the present paper to present such a treatment for the elimination of cotton anthracnose from infected seed.

TREATMENT OF INFECTED SEEDS WITH DRY HEAT

Within the last two decades, investigators interested in the control of plant diseases have attempted to eliminate seed-borne infection by the application of heat, hoping to discover a differential tolerance of the seed and the invading fungus. In this work, both dry and moist heat have been employed with varying degrees of success. In 1908, Kuhle[22] stated that he was able to free barley of loose smut infection by the use of dry heat, but was only partially successful in eliminating wheat smut. By first drying wheat and barley seeds for short periods at successively higher low temperatures then finally subjecting them to dry heat at 90° C. to 110° for ten minutes, Störmer[31] decreased greatly the amount of loose smut on barley, but accomplished very little decrease of wheat smut. Temperatures of 80-100° applied for 45 minutes resulted in little or no reduction of smut in summer wheat and summer barley.[32] In 1911, Appel and Riehm[1] treated smut-infected seeds of wheat and barley by soaking them for periods of 2 to 8 hours then heating them for 10 minutes in water at 48-50° C. In the case of the longer periods of soaking, the per cent of smut was markedly reduced. In other trials, it was found that better control was obtained by heating in water for 10 minutes at 54-56° C. No disease developed when seed were permitted to imbibe about 17 per cent of water and were then heated for 20 minutes in an oven at 55-60° C. In 1916, Naumov,[25] attempting to develop a treatment for cereal seeds infected with fungi (*Gibberella saubinetti*, *Fasarium roseum* and *F. subulatum*) believed to be the cause of the condition known in Russia as "intoxicating bread," heated rye at 66° C. and wheat, oats and barley at 60° C. for periods ranging from 24 hours to 3 days. He believed that by this method he was able to kill these seed-borne fungi without reducing the germinability of the seed. Atanasoff and Johnson,[2] however, in tests with scab of wheat and barley were unable to verify Naumov's experiments. However, when they subjected wheat, rye, oats and barley to treatment with dry heat at a temperature of 100° C. for 30 hours, they found that these seeds withstood the prolonged treatment remarkably well, the reduction of germination ranging between 10 and 40 per cent exclusive of a very few extreme cases. Moreover, seed-borne infections of the bacterial blight of oats and barley were killed and wheat scab and spot blotch of barley practically eliminated by this treatment. Also, the Helminthosporium blotch diseases and the loose smuts of oats and barley were greatly reduced. In 1922 Blodgett[3] heated Bliss Triumph potato tubers infected with mosaic in water at temperatures from 35 to 80° C. for varying times. He concluded that at the temperatures used the potatoes were killed in less time than was required to destroy the mosaic virus. In 1923 Schitikova[28] reported that dry heat at 60° C. to 66° C. for 24 to 48 hours and 85° for 30 minutes does not injure germination of wheat and is efficaceous against smut, but

does not completely suppress the disease. In the same year, Doran[18] reported that heating diseased seed of the garden bean for two hours at 80° C. did not give as good control of bean anthracnose as other methods.

The marked success of the dry heat treatment in the control of cereal diseases led the writer to test the efficiency of this method for the elimination of cotton anthracnose from infected seed. The great reduction in anthracnose resulting from the hot water treatment noted above gave additional hope that the application of dry heat might prove effective in the treatment of diseased seed. Certain preliminary tests were accordingly begun in the spring of 1920. While this work was in progress Barre[9] and his colleagues, Lipscomb and Ludwig, at the South Carolina Experiment Station made brief reports on the progress of their studies of the physical factors influencing the vitality of the anthracnose fungus carried by diseased cotton seed. In 1919 Lipscomb[9, 10, 11] reduced the water content of infected seed from 9 to 2 per cent by desiccation in a vacuum and was led to believe that this treatment killed the fungus. He also reported that the "fungus is killed more readily by a combination of heating and drying than by desiccation alone." In 1920, Lipscomb and Ludwig[10] working with different lots of seed and different varieties of cotton were unable to confirm the findings of the previous year. In the following year, Ludwig[11] concludes as the result of various treatments such as drying in a electrical seed drier and hot air oven, sterilizing and storing in various situations, that none of these methods may be reled upon to reduce the infection to any marked extent.

In 1923 Lipscomb and Corley[23, 24] believing that cotton seed could not be successfully freed of anthracnose by heating in air because of harmful oxidation of the fats and proteins described a method developed by themselves whereby seed were subjected to high temperatures in the absence of oxygen. A special glass tube was devised within which the diseased seed and a quanity of CaCl were sealed in a vacuum of 1 mm. or less or in an atmosphere of nitrogen. After they had been allowed to dry, these seed were heated in this tube at a temperature of 100° C. for 26 hours. No injury resulted to the seed and the anthracnose was completely suppressed. Likewise, heating at 110° C. for 10 hours did little or no injury to the seed but destroyed all anthracnose. Exposures for periods of 1 and 3 hours at 110° C. and 1 hour at 120° C. greatly diminished but failed to completely eliminate the disease. This method although effective has the disadvantage of requiring that the seeds be heated in a vacuum or in some inert atmosphere.

MATERIALS AND METHODS

This work began in the summer of 1920 with the use of seed from the 1919 crop. Owing to the tendency of the seed-borne inoculum to lose its viability after one year, a new supply of infected seed was obtained each season. Cook was the variety used in 1923 and 1924; the names of the varieties used in other years are unknown. Fifty per cent or more of the seeds of all lots used were shown by tests to carry viable inoculum of the anthracnose fungus.

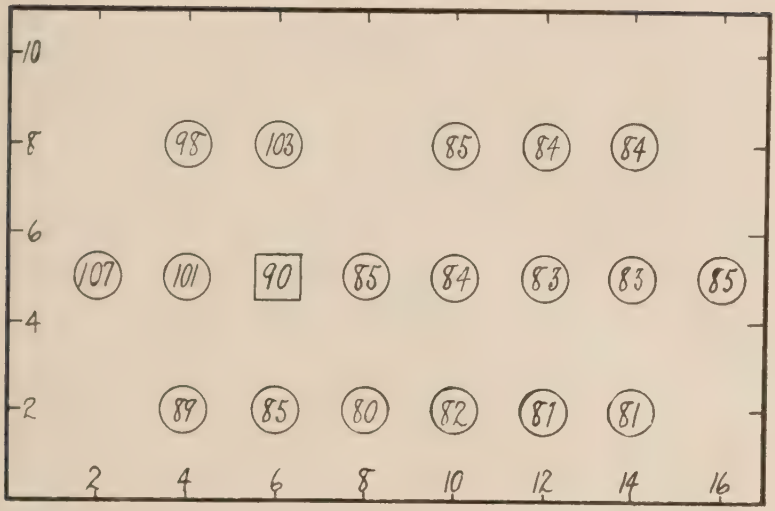


FIG. 1. Map showing the variations in temperature which obtain between various positions on the shelf of the electrical oven. The numbers in the circles are the temperatures at the positions indicated by the circles when the oven thermometer at the place marked by the square stands at 90° C. The marginal numbers represent inches.

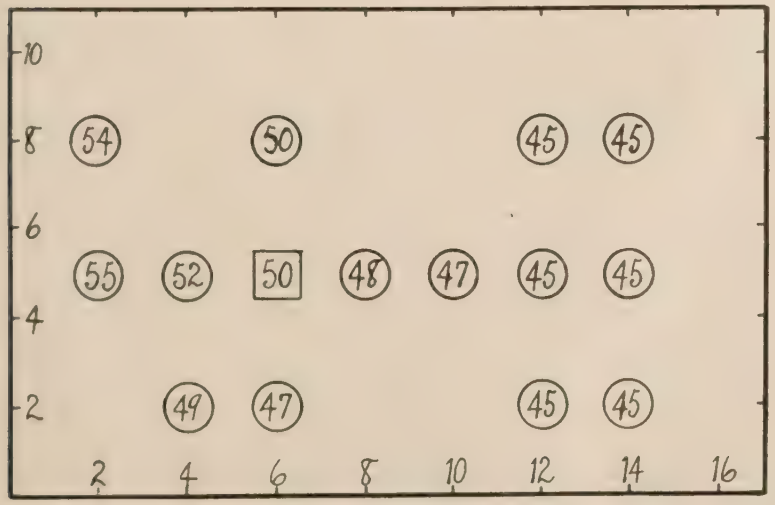


FIG. 2. Map showing the variations in temperature which obtain between various positions on the shelf of the electrical oven. The numbers in the circles are the temperatures at the positions indicated by the circles when the oven thermometer at the position marked by the square stands at 50° C. The marginal numbers represent inches.

With the exception of a few preliminary tests made in a gas oven, all lots of seed have been treated in an electric oven provided with a constant temperature regulator. This oven which has been kept in a constant temperature incubator room during fall, winter and spring, and in the open laboratory during the summer, has maintained itself within one degree of the desired temperature. During the tests with seed of the 1923 and 1924 crops, this oven was equipped with a temperature recording device in order that the operator should be informed of any deviation from the desired temperature due to temporary failure of the heating current. All tests were discarded in which significant variations from the desired temperatures had occurred. A considerable number of tests made in the early part of this study had to be discarded because of an unobserved source of error which made them untrustworthy and which should be avoided by persons desiring to do work requiring the use of definite temperatures. This source of error arises from the fact that the difference in temperature between two positions on the same oven shelf may be surprisingly large. This became apparent when several lots of seeds which had been heated concurrently but for different periods of time on the same shelf of the oven gave unexpected differences in percentage of germination. The lotes heated for the shortest periods showed strong reduction in germination while those heated for the longest periods showed no reduction. When the temperatures of the different positions on the oven shelf were tested it was found that there are differences of as much as 10° C. between certain positions which are not more than 2 inches apart. This situation is represented in Figs. 1 and 2, which show the temperatures at certain indicated positions on the oven shelf when the oven is being operated at two different temperature levels. The small square indicates the locus of the oven thermometer. The circles show the temperatures found at the positions of the other thermometers when the oven thermometer stands at the temperature recorded in the square. The temperatures at one end and the back of the oven are too high while those at the other end and the front are too low. Obviously, when several lots of materials are being heated at the same time, and, presumably, at the same temperature, they are actually being subjected to temperatures which may differ sufficiently to introduce serious errors. After this variation in the temperature of different positions on the oven shelf was discovered, the different lots were heated singly and always in the same place and a thermometer was kept in direct contact with the seeds in order to make certain that they were actually being subjected to the temperature desired.

As cotton seed comes from the gin, it contains, as a rule, a goodly number of seeds possessing defective embryos and consequently incapable of germination. The greater part of these imperfect seeds may be recognized by their light weight, small size, and shrunken coats. In order to exclude undue variations in percentage of disease and germination which might arise from the presence of faulty seeds, only such seeds were selected as appeared capable of germination.

Unless otherwise specified, fifty seeds were used in each lot and the percentage of diseased seedlings is based on the number of seeds in each lot.

Except where otherwise specified, all germinations to determine the percentage of seeds carrying viable elements of the anthracnose fungus have been made by the method recommended by Barre.[13] Small squares of blotting paper were loosely placed in test tubes to make a column about 3-4 inch deep. This was moistened with tap water and the tubes were plugged and autoclaved at 15 pounds for 20 minutes. By use of long, sterile forceps, the seeds were placed one in each tube. The forceps were flamed after handling each seed as a precaution aganist carrying inoculum from seed to seed and thus increasing the percentage of infected seedlings. During the time required for each test the tubes containing the germinating seeds were kept in a covered, galvanized iron box containing an open vessel of water to prevent excessive drying while the seeds were sprouting.

The first records were usually taken after the seeds had been in the germinator 6 to 8 days. The ungerminated seeds and healthy seedlings were returned to the germinator for another period of 5 to 7 days after which the percentages of disease and germination were again recorded. Often the tubes were put back, and records taken after a third period, thus extending the time in the germinator to 20 or more days. Usually, germination is complete and the maximum of disease has developed by the 15th day. A microscopic examination of each diseased seedling was made in order to ascertain the cause of its death. In addition to the anthracnose organism, other fungi, especially Fusarium, Macrosporium and bacteria often attack the seedlings.

EXPERIMENTAL

As a preliminary study, it seemed desirable at the outset to determine, as nearly as possible the temperatures most favorable for germination of cotton seed, for growth of the anthracnose fungus and for development of the disease on the seedlings growing in test tubes. Accordingly, experiments were planned to test the effect of temperature on these processes.

EFFECT OF TEMPERATURE ON GROWTH OF GLOMERELLA GOSSYPII

Equal quantities of Czapek's nutrient solution[27] solidified with 1.5 per cent agar and adjusted to a reaction of pH 7.2 were poured in petri dishes of uniform diameter. After the agar had solidified the dishes were inoculated at the center with a loopful of a suspension of conidia in sterile water. Three of these cultures were then placed in each of four ovens which were adjusted to maintain temperatures of 21, 26, 30 and 34.5° C. Growth at the different temperatures was measured comparatively by determining the diameter of the colony at stated periods and by estimating the relative densities of the colonies formed and the abundance of the conidia produced. Chaudhuri[16] working with *Verticillium albo-atrum* has shown that, while colony diameter is untrustworthy as a measure of the actual production of fungus material, it is satisfactory as a method of studying the relation of different temperatures to rate of growth in a medium of uniform composition and thickness.

TABLE II

Effect of Temperature on The Growth of *Glomerella Gossypii* on Nutrient Agar in Petri Dishes. (Increasing Abundance of Mycelium is Indicated by Number of † Marks. The Data Presented Represent The Average of Two Colonies in Each Case).

Exp. No.	Temp. Deg. C.	Diam. of Colony in mm.					Relative abundance at end of 4 Days		
		1 da.	2 da.	3 da.	4 da.	6 da.	Aerial Mycelium	Mycelium in Substratum	Conidia
1	21	No growth.	5.5	16.5	26.5	----------	†	†	†
1	26	4.0	19.0	30.5	45.0	----------	†††	††	†††
1	30	5.5	19.5	30.0	44.5	----------	††††	†††	†
1	34.5	No growth	No growth	No growth	No growth	----------	----------	----------	---------
2	25	----------	14.5	25.0	41.0	61.0	†††	††	††
2	27.5	----------	15.0	28.5	40.0	58.0	†††	†††	†††
2	30.5	----------	16.5	27.5	38.5	57.0	††††	††††	†
2	33 0	----------	8.5	10.0	15.0	22.0	††††	††††	†

A study of Table II, which presents the data of this experiment (Exp. 1), shows that temperatures of 26 and 30° C. are about equally favorable for growth with a possible slight balance in favor of 30°. Growth was less abundant at 21° C. and nil at 34.5° C. A temperature of 26° was most favorable for conidial production. A repetition of this test using potato glucose agar gave results concordant with those of the first test. The results of a second experiment which was similar to the first in every detail except that the ovens were maintained at temperatures of 25, 27.5, 30.5, and 33° C. are also presented in Table II. This test (Exp. 2) shows that only negligible differences in growth occurred at temperatures of 25, 27.5 and 30.5° C. Much less growth took place at 33° C. Conidial formation was much better at the two lower temperatures.

The tests described above may be summarized by stating that except for the highest, growth occurred at all temperatures used and was greatest in the range from 25 to 30.5. It would seem that with a given lot of inoculated seed the disease should develop most rapidly and on the greatest number of seedlings within this range of temperature.

EFFECT OF TEMPERATURE ON GERMINATION OF COTTON SEED

In order to determine what are suitable temperatures for germination of cotton seed, the following experiment was performed. Six hundred seeds were shaken in a quantity of sterile tap water until the fuzz was thoroughly wetted through to the seed coat. These seeds were then divided into 6 lots of 100 seeds and each lot was placed on moist blotting paper in a separate, covered, glass germinator. The germinators were kept at temperatures of 15, 20, 25, 30, 35 and 40° C. The percentage of germination was ascertained at short intervals, and the hypocotyls were measured and the average length computed at the end of 64 hours.

TABLE III

GERMINATION OF COTTON SEEDS AT DIFFERENT TEMPERATURES (100 SEEDS IN EACH LOT).

Lot No.	Temp. of Germinator Deg. C.	Per Cent of Germination After Hours Indicated					Av. Length of Radicle at end of 64 hrs. mm.	No. Seedlings Measured
		26	41	50	64	114		
1	15	0	0	1	31	48	None over 2 mm.	----
2	20	2	61	89	95	96	16.8	96
3	25	11	63	85	94	94	17.1	97
4	30	64	97	99	99	----	32.6	99
5	35	32	66	84	91	91	22.4	65
6	40	0	0	0	0	0	----	----

Table III, which presents the data obtained in this experiment, shows that, when judged by promptness and completeness, germination was decidedly most satisfactory in the lot held at 30° C. Very good and practically equal germination occurred in the two lots held at 20 and 25°, but these temperatures are obviously less favorable than 30°. At 35° germination was a little more prompt but less complete than at 20 and 25°. Sprouting was very slow at 15° and did not occur at all at 40°. When one compares the values found for the average length of the radicles, the different lots are again found to fall in the order given above indicating the relation of temperature to germination. The average length of the radicles of lot 4 was 32.6 mm., that of lot 5 was 22.4 mm., and that of lots 2 and 3 were 16.8 and 17.1 mm. The temperature of 35° is unfavorable for survival and continued growth of the seedling after germination. This is indicated by the fact that the seedlings die and decay much earlier at this than at lower temperatures. By the end of the 64 hour period in the germinator, 26 of the 91 seedlings had decayed to the extent that they were not used in obtaining the measurements.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF ANTHRACNOSE ON SEEDLINGS

Three tests were made to determine approximately the most favorable temperature for the development of anthracnose on cotton seedlings growing on moist blotting paper in test tubes. In the first test, disease-free seed of the Dixie variety were inoculated with a suspension of conidia and put into test tubes to germinate. One lot of 19 tubes was placed in each of four incubators set to run at temperatures of 20, 25.5, 30.5, and 35° C. In the second test, disease-free seedlings which had grown in test tubes for 13 days and which had reached a length of 5 to 6 cm. were inoculated by atomizing them with a suspension of conidia. They were then separated into 4 groups of 10 seedlings each and placed in incubators at temperatures noted above. In a third test, three lots of six-day old seedlings were atomized with a suspension of conidia and put to germinate at 18-25° (18° for 18 hours, 25° for 6 hours), 25.5, 30, and 33° C. Tables IV and V present the data derived from these three experiments.

TABLE IV

Effect of Temperature on The Daily Development and Percentage of Anthracnose on Seedlings Growing From Seeds Inoculated With A Suspension of Conidia of *Glomerella Gossypii*.

Exp. No.	Temp. Deg. C.	Days From Time Put to Germinate: 2	14	2	5	6	7	8	14
		Per Cent Germination		Per Cent Anthracnose					
1	20	60	60	0	0	0	10	60	60
1	25.5	60	60	0	30	60	60	60	60
1	30.5	70	70	0	70	70	70	70	70
1	35	90	90	0	0	0	0	0	0

TABLE V

Effect of Temperature on The Daily Development and Percentage of Anthracnose on Seedlings Atomized With A Suspension of Conidia of *Glomerella Gossypii*.

Temp. Deg. C.	Experiment No. 2: Days From Time Put to Germinate: 2	3	4	6	12	Experiment No. 3: Days From Time Put to Germinate: 2	3	4	6
20	0	0	0	30	70	0[a]	0[a]	10[a]	60[a]
25.5	0	0	60	70	70	0	80	100	100
30	0	30	50	60	60	0	100	100	100
33						0	60	60	90
35	0	0	0	0	0				

a Temperature of this lot varied between 18 and 25° (18° for about 18 hours and 25° for 6 hours).

It is evident that the temperatures of 25 and 30° C. are very near the optimum for the development of anthracnose on seedlings grown under the conditions of these tests. The disease appeared on most of the seedlings incubated at 20° C. but at this temperature it developed more slowly and did not destroy the seedling as quickly as at 25 and 30° C. This slow development of the disease at 20° is probably correlated primarily with the less rapid growth of the causal organism at that temperature. At 35° C., a temperature which inhibits growth of the fungus, anthracnose failed to develop.

From the results of the several temperatures experiments described above, the following conclusions applying to the particular conditions of the tests may be set forth:

1. Temperatures of 25 and 30° are not only very favorable for germination of cotton seed and the subsequent growth of the seedlings but also closely approximate the optimum for growth in culture of the fungus causing cotton anthracnose and for the development of the disease.

2. While a temperature of 35° C. permits good germination of cotton seed, it inhibits the growth of the fungus which causes anthracnose and prevents the development of the disease.

3. At 20° germination of cotton seed and growth of the seedlings are good, but growth of the anthracnose fungus and the development of the disease are slower than at higher temperatures.

4. Temperatures between 25 and 30° C. are to be regarded as most favorable for the germination of cotton seed when it is desired to determine the percentage of infection from anthracnose by the method described in this paper. At these temperatures germination of the seeds is rapid and complete and timely development of anthracnose on inoculated seedlings is assured.

DRY HEAT TREATMENT OF COTTON SEED FOR CONTROL OF ANTHRACSE

EXPERIMENTS WITH SEED IN SMALL LOTS

The experimental work bearing directly on the control of cotton anthracnose was begun in a preliminary way by subjecting air dry cotton seed to temperatures of 70, 80, 90, and 100° C. and subsequently determining their ability to germinate. The data obtained in many of these preliminary tests later proved to be of doubtful value and had to be discarded, as noted above, because of the great variation in temperature which obtains between different positions on the same shelf of the oven used for heating the seeds. Inasmuch as several lots were usually heated concurrently, and no record of the position of the individual lot in the oven was kept, it was impossible, when this error was discovered, to tell, even by aid of the temperature map of the oven, the exact temperature to which a given lot had been subjected. Since differences of 10 to 15 degrees existed between lots heated at the same time, it was necessary to repeat many of the earlier tests, placing the seed only in positions known to have the temperatures indicated by the oven thermometer. Table VI gives the data obtained from such of the preliminary tests as are known to be trustworthy and from others made later in repetition of those which were discarded.

TABLE VI

RESISTANCE OF AIR DRY COTTON SEED TO TEMPERATURES 70, 80, 90 AND 100° C.

Exp. No.	Lot No.	Temp. Deg. C.	Time Hrs.	Per Cent Germination		Water Content of Seeds in Per Cent of Wet Wt.	Year When Seed Were Grown
				Treated	Untreated		
1	1	100	¼	0	100	----------	1919
2	1	90	¼	0	100	----------	1919
3	1	80	1	34	98	----------	1921
3	2	80	4	24	98	----------	1921
3	3	80	15	12	98	----------	1921
4	1	80	1	5	98	----------	1922
5	1	70	6	70	90	----------	1919
5	2	70	24	40	90	----------	1919
5	3	70	72	60	90	----------	1919
6	1	70	24	98	92	8.3	1923
6	2	70	48	90	92	8.3	1923
7	1	70	24	80	97	14.3	1923
7	2	70	24	78	97	11.0	1923
8	1	70	24	73	71	7.98	1924
8	2	70	24	60	72	10.65	1924
8	3	70	24	49	77	13.22	1924

It early became evident that cotton seed in an air dry condition cannot endure the high temperatures used by Atanasoff and Johnson[2] and others in the treatment of cereal seeds. Air dry cotton seeds completely lost their power to germinate when heated at temperatures of 90 and 100° C. for periods as short as 15 minutes. The application of dry heat at 80° C. for one hour completely inhibited germination of seed of the 1919 crop and greatly reduced and prolonged germination of the 1921 and 1922 crops. Dry heat at 70° C. was endured for much longer periods without total loss of viability but even that temperature produced marked injury to certain lots of seed. In Experiment 5, in which three lots of seed of the 1919 crop were heated at 70° C. for 6, 24 and 72 hours, the treated seed yielded germination percentages of 70, 40 and 60 in comparison with 90 for the untreated check seeds. Other tests with seed of this crop yielded similar results. In a test (Exp. 6) with seeds of the 1923 crop whose water content had diminished to 8.3 per cent as a result of storage under laboratory conditions, two lots which had been heated for 24 and 48 hours gave respectively 98 and 90 per cent of germination in comparison with 92 per cent for the untreated seeds. In Experiment 7, two lots of seeds of the 1923 crop taken from the same container as those used in Experiment 6 were stored over water in a closed dish until they had absorbed sufficient vapor from the moist air to raise their water content to 11 and 14.3 per cent; then each lot was heated for 24 hours at 70°. A marked reduction in the percentage of germination resulted from the treatment. Seventy-eight and 80 per cent of the seed of the two treated lots and 97 per cent of the untreated lot germinated. In Experiment 8, three lots of seed of the 1924 crop were used. These seed originally came from the same bag but differed in water content as a result of different storage conditions for several days previous to the time of the heat treatment. Lot 1 was stored in an open dish in the laboratory, and underwent a marked reduction of its water content; lot 2 was taken from the center of a two bushel bag stored in the laboratory and had lost less water because of the bulk of the seed around it, and lot 3 was kept in a glass stoppered specimen jar. The three lots contained water amounting respectively to 7.98, 10.65 and 13.22 per cent of their wet weight, and after being heated at 70° for 24 hours, gave germination percentages of 73, 60 and 49 in comparison with 71, 72, and 77 for corresponding unheated lots. Seventy degrees appears to be slightly above the maximum temperature which cotton seed containing an amount of moisture normal to ordinary storage conditions can safely endure for any considerable period of time.

A number of tests were made to determine the amount of anthracnose which might develop on seedlings growing from seeds which had been heated at 70°. Table VII shows the results of some of these tests.

TABLE VII

PER CENT OF COTTON SEEDLINGS INFECTED WITH ANTHRACNOSE GROWING FROM SEED PREVIOUSLY HEATED AT 70° C.

Exp. No.	Lot No.	Duration of Treatment, Hrs.	Per Cent Anthracnose	
			Treated Seed	Check
1	1	1	44	
2	1	24	2	20
2	2	48	1	20

When seed had been heated at 70° for one hour, 44 per cent of the seedlings developed anthracnose. After seed had been heated 24 hours, the percentage of seedlings which became diseased was reduced from 20 per cent in checks to 2 per cent in the treated. 48 hours at 70° C. did not suffice to completely free the seeds of anthracnose. Manifestly, a dry air temperature of 70° C. causes a great reduction in the amount of anthracnose appearing on seedlings from diseased seed, but fails to eliminate the disease within a period of time sufficiently short to obviate injury to seed containing a normal amount of water.

When it became apparent that the application of temperatures of 70, 80, 90 and 100° to cotton seed for periods of time sufficiently long to destroy the anthracnose fungus brought about marked reduction of germinative power of the seed, tests were made to determine whether decrease of the normal water content by desiccation at temperatures too low to be harmful would increase the resistance of the seeds to high temperatures. Accordingly, cotton seed having a normal air dry water content was stored over H_2SO_4 or CaO_2 in desiccators or dried in ovens at temperatures of 40, 50 or 60° C. for varying periods of time. After this preliminary desiccation, the seed were heated at temperatures of 60, 70, 80, 90 or 100° C. for certain periods of time. Such of these tests as deal only with water relations of treated seeds will be described and the results given in detail in a later section, Part II, of this paper. It will suffice to state at this point that drying of seeds at low temperatures enables them to endure for long periods of time temperatures which otherwise would have been quickly destructive. The following paragraps will be devoted to a consideration of the effect of this treatment on the viability of the seed-borne elements of the cotton anthracnose fungus. A large number of experiments have been made with seed grown in six different seasons. These tests are grouped, in accordance with the particular high temperature used and the results are set forth in appropriate tables.

Table VIII presents the data derived from a number of tests in which highly infected cotton seed were dried in ovens at 40-45° C and subsequently heated at 60° C. for varying periods of time.

TABLE VIII

Per Cent of Anthracnose and Germination in Cotton Seed Which Has Been Subjected to Drying at 40-45° C. and Subsequent Heating at 60° C.*

Exp. No.	Lot No.	Time of Preliminary Drying at 40-45° C. Hours	Time of Heating at 60° C. Hours	Per Cent of Seeds Developing Diseased Seedlings		Per Cent of Seed Which Germinated	
				Treated	Untreated	Treated	Untreated
1	1	72	48	2		86	
	2	72	72	0		80	
	3	72	96	2		82	
2	1	72	Not heated	14	40	96	96
	2	72	24	2	40	90	96
	3	72	48	4	40	92	96
	4	72	72	2	40	86	96
3	1	72	24	4		96	
	2	72	48	2		90	
	3	72	72	2		94	
4	1	24	72	0	0	98	98
5	1	144	Not heated	4	40	92	96
	2	120	48	8	40	86	96

*Seed of the 1919 crop was used in all tests except Exp. 4 in which case seed was from the 1920 crop. In age the seed ranged from 10 months in Exp. 1 to 15 months in Exp. 3.

When comparison of the percentages of diseased seedlings derived from treated and untreated seed is made, it is obvious that this treatment greatly reduces but does not entirely eliminate seed-borne infection. Forty per cent of the seedlings from untreated seed developed anthracnose but not over 4 per cent from the treated seed became diseased. The preliminary drying at 45° reduced the number of diseased seedlings to 14 per cent in 72 hours and to 4 per cent in 144 hours. Strangely, heating at 60° for 24 hours seems as effective as the 72 and 96 hour period. Apparently the fungus in or on a very small percentage of the seeds is much more resistant than in the rest of the seeds. Experiment 4 shows that a preliminary drying for as short a time as 24 hours fully prepared the seed to endure 60° C. for 72 hours. A number of tests not here tabulated indicate that as a result of ageing, the disease had completely disappeared from the seed used in Experiment 4 by the time that experiment was made.

When it was learned that a temperature of 60° C. was not sufficiently high to kill all seed-borne infection in a reasonable time, a number of experiments were made using a temperature of 70° C. As before, the seed were given a preliminary drying at 40-45° C. Seed of the 1919, 1920 and 1921 crops, all heavily infected, were used in the experiments. Table IX sets forth the data pertaining to these tests.

TABLE IX

PER CENT OF ANTHRACNOSE AND GERMINATION IN COTTON SEED WHICH HAS BEEN SUBJECTED TO PRELIMINARY DRYING AT 40-55° C. AND SUBSEQUENT HEATING AT 70° C.*

Exp. No.	Lot No.	Temp. of Prelim. Drying Deg. C.	Time of Prelim. Drying Hours	Temp. of Heating Deg. C.	Time of Heating Hours	Per Cent of Seeds Developing Diseased Seedlings		Per Cent of Seeds Germinated	
						Treated	Untreated	Treated	Untreated
1	1	45–50	72	Not heated	Not heated	22	----------	84	----------
	2	45–50	72	70–75	24	6	----------	95	----------
	3	45–50	72	70–75	48	0	----------	100	----------
2	1	45–50	72	67–74	24	0	0	92	94
	2	45–50	72	67–74	48	4	0	96	94
	3	45–50	72	67–74	72	0	0	90	94
3	1	50–55	24	70–73	24	2	6	98	98
	2	50–55	24	70–73	48	0	6	100	98
	3	50–55	24	70–73	72	0	6	94	98
4	1	40–45	48	70	72	0	----------	90	----------
5	1	40–45	24	70	72	0	10	88	98
6	1	40–45	24	70–73	48	50	86	84	96
	2	40–45	24	70–73	72	6	86	92	96
7	1	40–45	24	70–73	72	24	64	92	94

*In Experiments 1 to 3, seed of 1919 crop was used; in Experiments 4 and 5, seed of 1920 crop; in Experiments 6 and 7, seed of 1921 crop. Experiments 1 to 3 were done in a gas oven. Seed used in Experiments 6 and 7 was 4 months old; that in Experiments 2 and 3 was 13½ months old.

It is evident that even after the seed have been dried at 45° C. a temperature of 70°C. is not sufficiently high to kill anthracnose in every instance even when the treatment is prolonged to 72 hours. All the tests except Experiments 6 and 7 were made at a time when the fungus was losing its viability from ageing as indicated by the behavior of check lots of untreated seed. Seed used in Experiments 1-5 was 10 to 16 months old when treated, while that of Experiments 6 and 7 was only 4 months old. The treatment proved to be much less effective with the young four months old seed of the 1921 crop than with the 10 to 16 months old seed of the 1919 and 1920 crops. Germination of treated and untreated seed are seen to compare favorably.

Rather than prolong the heating at 70° C. beyond a 72 hour period, it seemed better to try the effects of higher temperatures. In these, as in previous tests, the seeds were subjected to a preliminary desiccation before applying the higher temperature. The results of these tests, are presented in Table X.

TABLE X

Per Cent of Anthracnose and Germination in Cotton Seed Subjected to Preliminary Drying at 40 to 50° C. and Subsequent Heating at 80° C.*

Exp. No.	Lot No.	Temp. of Prelim. Drying Deg. C.	Time of Prelim. Drying Hours	Temp. of Heating Deg. C.	Time of Heating Hours	Per Cent of Seeds Developing Diseased Seedlings		Per Cent of Seeds Germinated	
						Treated	Untreated	Treated	Untreated
1	1	50	24	80	24	10		96	
	2	50	24	80	48	12		94	
2	1	40-45	48	80	48	22		98	
	2	40-45	48	80	72	0		94	
3	1	40-45	24	80	72	0	34	80	98
4	1	40-45	48	80	72	0	46	86	98
5	1	40-45	24	80	48	2	54	88	90
	2	40-45	24	80	72	0	54	88	90
6	1	40-45	24	80	48	4	64	94	94
	2	40-45	24	80	72	0	64	92	94
7	1	40-45	72	80	24	30	64	94	94
	2	40-45	72	80	48	2	64	96	94
8	1	40-45	48	80	48	2		96	
9	1	50	12	80	72	2		98	

*In Exp. 1-4, seed of the 1920 crop was used; in Exp. 5-8, seed of 1921 crop; in Exp. 9, seed of 1923 crop. The age of the seed used ranged from 3 to 10½ months.

At the temperature of 80° C. the seed-borne infection is seen to be greatly reduced by the end of 48 hours and completely devitalized in every test except one by the end of 72 hours. The 12-hour period of preliminary desiccation at 50° C. and the 24-hour period at 40-45° and 50° are as effective as the 48-hour period in preparing the seed to resist the high temperature of the different periods of heating. Prolonging the period of drying to 72 hours does not make it possible to shorten the period of heating to 48 hours.

In order to render this method of seed treatment more economical of time and heat, it seemed advisable to determine whether the effective period of heating at 80° C. namely, 72 hours, could be shortened by the use of a higher temperature. Accordingly, a number of tests were made in which infected seed were subjected to a treatment consisting of a preliminary desiccation of 40-45° C. or 50-53° C. immediately followed by a period of heating at 90° C. The results of these tests are presented in Table XI.

TABLE XI

PER CENT OF ANTHRACNOSE AND GERMINATION IN COTTON SEED WHICH HAS BEEN SUBJECTED TO PRELIMINARY DRYING AT 40 TO 60° C. AND SUBSEQUENT HEATING AT 90° C.*

Exp. No.	Lot No.	Preliminary Drying		Heating		Per Cent of Seeds Developed Diseased Seedlings		Per Cent Germination	
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Treated	Untreated	Treated	Untreated
1	1	40–45	48	90	48	0	34	56	98
	2	40–45	48	90	72	0	----------	22	----------
2	1	40–45	24	90	24	0	----------	54	----------
	2	40–45	24	90	48	0	----------	70	----------
3	1	40–45	24	90	6	30	----------	88	----------
	2	40–45	24	90	13½	0	----------	80	----------
4	1	50–53	24	90	10	14	----------	100	----------
	2	50–53	24	90	14	2	----------	94	----------
5	1	50–53	24	90	18	0	----------	82	----------
	2	50–53	24	90	24	0	----------	78	----------
6	1	50	24	Not heated	Not heated	90	88	94	90
	2	50	24	90	18	4	88	94	90
7	1	50	24	90	18	0†	----------	93	----------
8	1	50	24	90	24	0	----------	96	----------
9	1	40	24	90	18	0	16‡	36	92
	2	45	24	90	18	2	16	62	92
	3	50	24	90	18	0	16	96	92
	4	55	24	90	18	0	16	96	92
	5	60	24	90	18	0	16	98	92
10	1	50	18	90	24	0	42	68	96
	2	50	18	90	24	0	----------	88	----------
11	1	50	18	90	20	1†	82	97	100
12	1	50	12	90	24	2	----------	62	----------
	2	50	18	90	24	0	----------	92	----------
	3	50	24	90	24	0	----------	96	----------
	4	50	36	90	24	0	----------	96	----------

*Seed of 1920 crop was used in Exp. 1; 1921 crop in Exp. 2-5; 1922 crop in Exp. 6-9; 1923 crop in Exp. 11-12. The age of the seed varied from 1 to 12 months.

†100 seeds in this lot; 50 seeds in all others not so marked.

‡The seed used had been delinted for 30 min. in H_2SO_4.

A study of Table XI shows that the anthracnose fungus was killed in six and greatly reduced in amount in the remaining two of the eight tests in which the seed were heated at 90° C. for 18 hours. The disease was completely eliminated in eight of the nine tests in which seed were heated for 24 hours and failed to develop in any test when the seed had been treated for as long as 48 hours. A relatively large proportion of the infective material survived the 6 and 10-hour periods.

The low percentages of germination in Experiments 1, 2, and 9 (Table XI) show that a preliminary drying at 40-45° C. for 24 and 48 hours does not well prepare cotton seeds to endure a temperature of 90° C. for any considerable length of time. When they had been given a preliminary treatment at 50° for 24 hours (Exp. 5, 8, 9, and 12), little or no reduction in germination occurred as a result of heating at 90°.

In an effort to further reduce the time required for this treatment, a number of tests were made using 100° C. as the major temperature. Seeds harvested in 1921 and 1922 were dried for 24 hours at 40-45° C., or at 50° C. then heated at 100° for periods varying from 2 to 24 hours. The results of these tests are embodied in Table XII.

TABLE XII

Per Cent of Anthracnose and Germination in Cotton Seed Which Has Been Subjected to Preliminary Drying at 42-63° C. and Subsequent Heating at 100° C.*

Exp. No.	Lot No.	Preliminary Drying		Final Heating		Per Cent of Anthracnose		Per Cent Germination	
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Treated	Untreated	Treated	Untreated
1	1	40-45	24	100	11	0		22	
	2	40-45	24	100	24	0		50	
2	1	50	24	100	2	6		32	
	2	50	24	100	4½	0		6	
3	1	50	24	100	6			40	
4	1	50	24	100	12	0	38	68	92
	2	50	24	100	6	0		58	
5	1	60	24	100	6	0	20	84	92

*Seed of the 1921 crop was used in Exp. 1-3; 1922 crop in Exp. 4; 1923 crop in Exp. 5.

A study of the data presented in this table shows that anthracnose developed on the seedlings in only one test, and in this instance, the seed had been heated only 2 hours. Inasmuch as germination was greatly reduced in all the tests after heating the seeds at 100° C., attempts to control anthracnose by use of this temperature were for a time discontinued. Several months later, after the encouraging results reported below had been obtained in the experiments in which temperatures at 60 and 95° were employed, another test was made in which 15 month old seed of the 1923 crop were heated at 100° for 6 hours. These seeds had been previously dried at 60° for 24 hours. Upon germination, none of the seedlings from treated seeds developed anthracnose (Table XIII, Exp. 5) while 20 per cent of those from untreated check seeds became diseased. Germination was appreciably but not excessively reduced by the treatment, 84 per cent of the heated seeds surviving the treatment.

The data embodied in Table XIII were obtained in a large number of tests in an effort to get better control of anthracnose than was obtained by heating seeds at 90° C. and still to obtain higher germination than seemed possible by the use of 100° C. In tests 1 to 6 the seeds were dried at 50° C. for periods of 12 to 36 hours, and in tests 7 to 9 the preliminary drying was done at 60° C. for 12 to 24 hours. The different lots were then heated at 95° C. for periods varying from 5 to 24 hours. In tests 8 and 9 the oven was provided with a recording instrument which made a continuous record of the temperature of the oven for the entire period of the treatment. The records of this instrument show that while the several lots of seed of these two experiments were being treated, no serious fluctuation of temperature occurred.

TABLE XIII

PER CENT OF ANTHRACNOSE AND GERMINATION IN COTTON SEED WHICH HAS BEEN SUBJECTED TO DRYING AT 50 TO 60° C. AND SUBSEQUENT HEATING AT 95° C.*

Exp. No.	Lot No.	Preliminary Drying		Subsequent Heating		Per Cent Anthracnose		Per Cent Germination	
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Treated	Untreated	Treated	Untreated
1	1	50	24	95	13	0	----------	90	----------
2	1	50	18	95	24	0	----------	64	98
	2	50	24	95	24	0	----------	72	98
3	1	50	18	95	12	0†	82	84	100
4	1	50	24	95	12	0	42	56	96
	2	50	24	95	12	0	----------	84	----------
5	1	50	24	95	7	0	----------	56	----------
	2	50	24	95	10	0	----------	84	----------
	3	50	24	95	5	0	----------	74	----------
	4	50	24	95	12	0	----------	46	------
6	1	50	12	95	8	0	----------	38	----------
	2	50	18	95	8	0	----------	68	----------
	3	50	24	95	8	4	----------	80	----------
	4	50	36	95	9½	0	----------	96	----------
7	1	60	24	95	10½	0†	----------	95	----------
	2	60	24	95	12	0†	----------	98	----------
8	1	60	24	95	11½	0†	26	95	----------
	2	60	24	95	8	1†	----------	97	
9	1	60	6	95	12	0	----------	20	
	2	60	12	95	12	0	----------	86	
	3	60	18	95	12	0	----------	94	
	4	60	24	95	12	0	----------	96	

*Seed of 1922 crop was used in Exp. 1 and 2; 1923 crop in Exp. 3-9. At the time of making the treatment the seed used in Exp. 1 and 2 was 12 and 13 months old, that in Exp. 3-9 varied in age from 2 to 6 months.

†100 seeds. 50 seeds in all lots not so marked.

As shown by table XIII anthracnose developed on seedlings from only two lots of treated seed, and in these two instances, namely Exp. 6, lot 3, and Exp. 8, lot 2, the seed had been heated for only 8 hours. In all the remaining lots of treated seed, some of which were heated for less than 8 hours, the viatality of the anthracnose fungus was completely destroyed while the untreated check lots yielded a high percentage of diseased seedlings.

The germination of the seed treated in Experiments 1-6 lot 3 was in most cases seriously reduced in comparison with that of the checks. Obviously, the heating of cotton seed containing a normal amount of moisture at 50° for 24 hours or less does not prepare the seed to withstand temperatures of 95° C. for any considerable period without marked loss of viability. When the seed are heated at 50° for 36 hours (Exp. 6, lot 4) or at 60° for 18 and 24 hours (Sxp. 7, 8, and 9, lots 3 and 4), excellent germination followed the subsequent treatment at 95° for 8 to 12 hours.

EXPERIMENTS WITH SEED IN BULK

As soon as it began to appear from the results of the tests made with seed in lots of 50 or 100 that control of anthracnose might be obtained by the application of dry heat for appropriate periods of time, experiments testing the efficiency of this method in the treatment of larger quantities of seed were begun. Use was made in a number of these tests of small gas and electric ovens such as are ordinarily found in plant pathological laboratories. In some of the tests the seed were spread out on the oven shelf; in others,

they were held in shallow baskets made of window screen. When a 7 x 11 inch basket containing a quart of seed was heated on the shelf of an electric or gas oven of such size as to leave a margin of 2 to 3 inches for circulation of air, the seeds in the bottom of the pan always became very much hotter than those on top and were killed before those on top reached a temperature effective for destroying the anthracnose fungus.

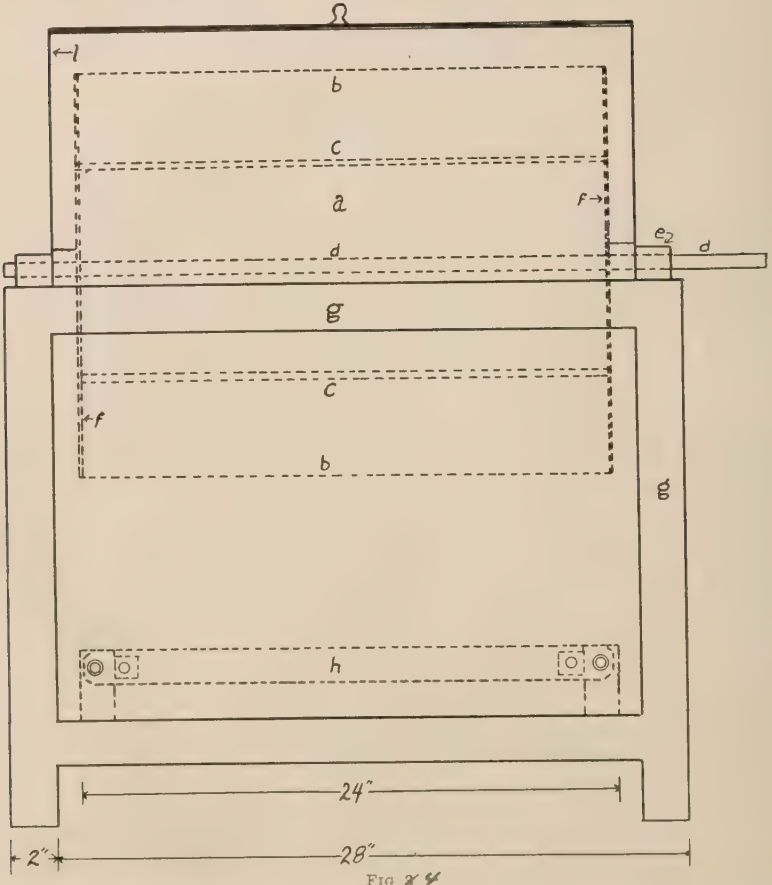
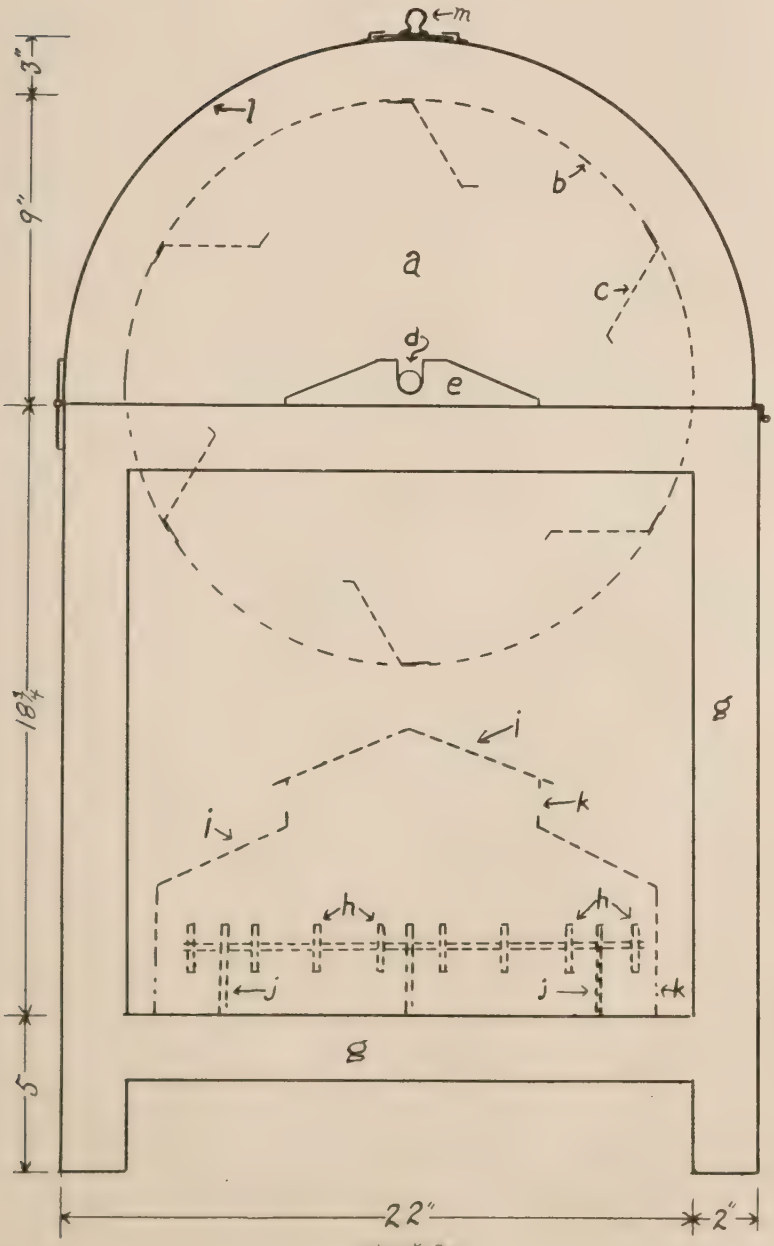


Fig 3 & 4

Figs 3 and 4. Drawing to illustrate the general structure of the seed treating machine. Fig. 3, an end view; fig. 4, a side view from the front. A, seed drum; b, ⅛ inch mesh wire netting covering seed drum; c, metal shelves which serve to stir seed as drum rotates and to which the wire netting is attached; d, shaft which forms the axle of the seed drum and which is connected to the speed reducing gears; e, bearing for axle of seed drum; f, end of seed drum; g, angle iron frame; h, electric heaters; i, shield to prevent lint from falling on heaters; j, supports for heaters; k, apertures to facilitate circulation of air; l, galvanized iron hood; m, slide for regulating ventilation.

In other tests, a larger oven was used with a tier of 5 baskets 14 x 9 x 1½ inches, each containing a quart of seed. These baskets, kept apart by putting ½ inch wooden strips across the top were stacked in such a position as to leave a marginal space of 1 inch at the sides and 1½ inch


m
3"
9"
l
b
a
c
d
e
18¼
g
i
k
i
h
h
j
j
k
g
5
22"
2"

at the ends for circulation of air. This too was unsatisfactory since great differences of temperature were found to prevail between the different baskets, the thermometers in the different baskets from the bottom upward showing 125, 110, 93, 81 and 75° C.

A larger oven was improvised from an old seed incubator which had shelves in the form of wire bottomed pans 21 inches square and 3 inches deep. About one peck of seed was heated at a time on one of these shelves, the seed being stirred at frequent intervals during the period of heating. Although by this procedure a much more uniform temperature could be maintained throughout the mass of seed, the results were unsatisfactory, both from the standpoint of regulation of the temperature and control of the anthracnose disease. Moreover, the method demanded the almost constant attention of the operator.

The difficulties which must ordinarily be overcome when one attempts to treat seed of any sort with dry heat are accentuated by the presence of the fuzz commonly found on cotton seed. When cotton seed is heated in bulk, the layers of fuzzy seed on the outside very effectively insulate the seed within the mass. When it was found that diseased seed could not be satisfactorily treated in bulk in ordinary gas and electric ovens, it was decided to build a special machine for the purpose. Accordingly, a machine which should keep the seed agitated while it is being heated was designed by the writer and built by Mr. V. W. Busbee, formerly of the Department of Mechanical Engineering. This seed treating machine is pictured in Plates I, II, and the construction is illustrated in Figs. 3 and 4. The rectangular body of the machine is 30 in. long, 18¾ in. tall and 22 inches wide and consists of a framework of heavy angle iron (g) welded into one piece. Asbestos board, 5/16 inch, is bolted to the iron framework to form the sides and ends, and heavy galvanized iron sheeting is used to form the bottom. One end is hinged to make a tightly fitting door. The corner angle irons extend 5 inches below the bottom to serve as legs. The semi-cylindrical hood or top (1) is composed of heavy sheet iron lined with sheet asbestos and is hinged so that it may be swung back out of the way when the machine is being loaded, unloaded or cleaned. A double row of ¾ inch holes in the bottom and a single row of 10 one-inch holes in the top provided with sliding shutters (m) serve to regulate the ventilation. The two upper end pieces of the angle iron frame carry bearings (e) for a shaft which supports a cylindrical drum (a) for holding and agitating the seeds while they are being heated. This drum is 24 inches long by 18 inches in diameter. The ends are made of heavy metal to which galvanized wire screening (b) having 8 meshes to the inch is fastened to form the curved sides. This screening is also fastened to flanges along the outer edges of 6 metal shelves (c) which are fastened between the ends of the cylinder in such a position as to lie in the plane of the radii of the cylinder. As the cylinder revolves, the shelves raise the seed from the bottom to near the top of the cylinder, and, while making this journey, the seed slide gradually off the shelf, falling to the bottom of the cylinder again. Thus the seeds are being constantly mixed and stirred and the hot air is kept circulating among them while the treatment is in progress. A small section of the side is so

fitted as to be readily removed for loading and unloading the drum. The shaft (d) which passes along the longitudinal axis of the drum is fastened at one end to a system of reducing gears which in turn is connected by a belt to a small motor of $\frac{1}{8}$ horsepower. By means of the reducing gear, the speed is reduced from 1800 revolutions per minute at the motor to about $1\frac{1}{2}$ revolutions per minute at the drum containing the seeds.

The heat is supplied by 8 C-H electric space heaters* (h) placed in the bottom of the machine and directly under the drum for the seeds. In order to prevent lint and sand from accumulating on the heaters, a ventilated removale shield (k i) is inserted between the heaters and the seed in the drum. The temperature is regulated automatically by means of a thermostat which controls a circuit-breaker.

The sensitive element of the thermostat was made by firmly riveting together two strips of iron and zinc to make a tongue of metal 11 inches long and tapering gradually from 1 inch at the widest end to $\frac{3}{8}$ inch at the tip. This strip of metal was bent into the shape of a narrow letter U with one side twice as long as the other. The end of the short arm was attached to a metal base and a point of platinum metal was attached to the tip of the long arm. A second platinum metal point was fastened into the tip end of an adjustable screw and the two parts bearing the metal points were set in such relative positions that when the temperature of the oven arose or fell, the two points made or broke contact respectively. The adjustable screw made it possible to increase or diminish the distance between the two points and thus to regulate the temperature of the oven. The thermostat is fastened to the inner surface of the front asbestos wall of the oven and suitably insulated wire connects its two parts to proper poles on the circuit breaker.

The circuit breaker (Fig. 5) is mounted on a slate base. It consists of two glass cups (e) containing a small amount of mercury and two electro magnets (d) capable of raising or lowering a U-shaped piece of metal (g). Tinned iron nails dipping into the cups of mercury are connected to the source of power and the bar of metal carries a short copper wire (f) to the ends of which other tinned iron nails are soldered. These nails are so adjusted in height above the mercury surface in the cups that when current is flowing through the thermostat they are held out of the mercury, but when the oven temperature falls and current ceases to flow through the thermostat, their points drop into the mercury cups, thus closing the heating circuit. A signal light burns when current is flowing through the heaters. Since the current which flows through the thermostat and operates the circuit breaker is taken from the same source as that which supplies the heaters, no batteries are needed. A condenser is connected across the contacts of the thermostat to reduce arcing at that point and consequent clatter of the circuit breaker. The motor which rotates the drum is operated independently of the heating circuit. The wiring diagram is shown in Fig. 5.

Thermometers inserted through the walls of the machine have made it possible to obtain a knowledge of the temperatures obtaining at different places in the machine while the seeds are being treated. In practice, it is

*Manufactured by Cutler-Hammar Mfg. Co., New York.
Foot-note page 55.

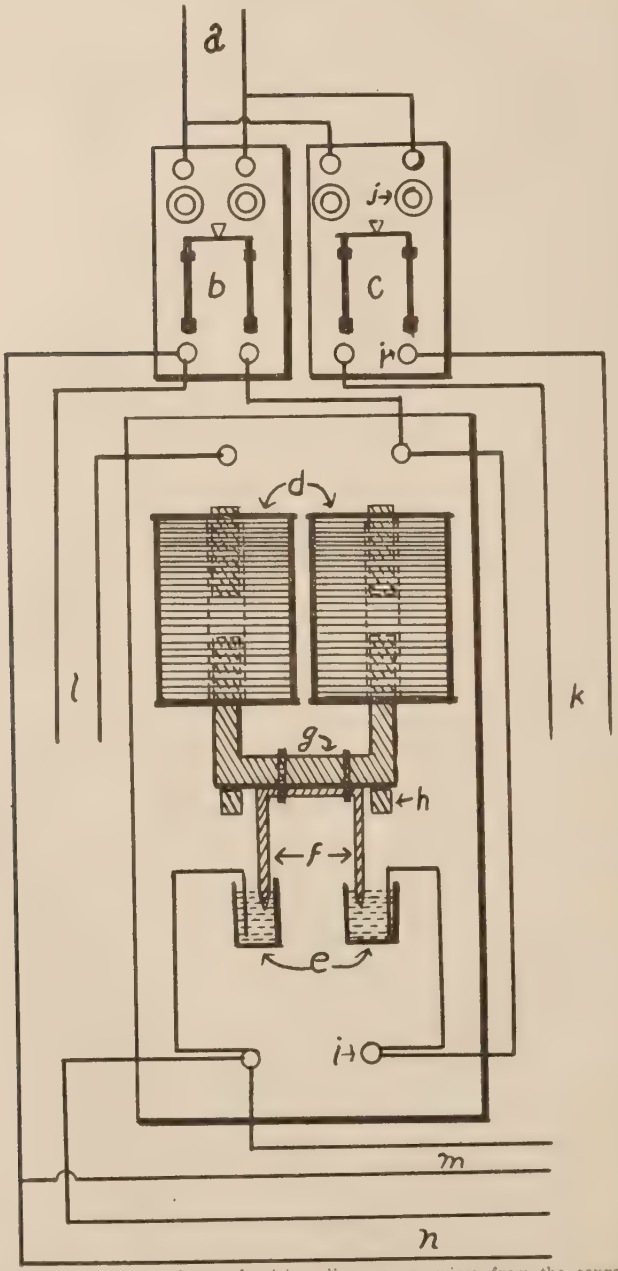


Fig. 5. The circuit breaker and wiring diagram; a, wires from the source of electrical supply; b, switch in heating circuit; c, switch in motor circuit; d, two electro magnets; e, glass cups containing mercury; f, conductor which connects the two mercury cups when current is flowing through heaters and which is raised out of mercury when the electro magnets raise the armature; g; h, electric heaters; i, shield, to prevent lint from falling on heaters; j, supports for heaters; k, apertures to facilitate circulation of air; l, galvanized iron hood; m, slide for regulating ventilation

best to rotate the seed cylinder so that it comes up toward the front and descends at the back of the machine. The motion of the cylinder then directs the current of hot air toward the front of the machine where it strikes the thermostat. Since the seed are being carried toward the front of the machine and upward in the rotating drum, they are being heated by the same current of hot air which is playing upon the thermostat. At the level of the thermostat the temperature at the back of the oven is 10 to 15 degrees lower than at the front owing to the direction in which the air in the oven is circulating. The temperature at the top of the hood just above the cylinder is commonly 1 to 3 degrees C. lower than on the level of the thermostat in the front of the machine. In practice, the temperature shown by the thermometer inserted through the front right hand corner (Thermometer A) of the frame is taken to indicate the temperature of the seed: As indicated by this thermometer there is a variation of ½ to 1° C. between the temperatures at which the thermostat closes and breaks the heating circuit. After the heating circuit breaks, the temperature continues to rise 1 to 1½° C. and after the circuit closes, the temperature continues to fall 1 to 1½° C.; so including this lag there is a total variation of air temperature in the oven ranging from 2½ to 4° C. as indicated by thermometer A.

Some of the first lots of seed treated in this machine were ruined because of defective operation of the circuit breaker and thermostat. Modifications have been made which obviated these earlier defects so that the machine now operates in a very reliable manner. After the thermostat has been adjusted to give the temperature desired, it is necessary to visit the machine only occasionally to oil the bearings.

The total spatial capacity of the seed drum is 2.6 bu., after making liberal allowance for space occupied by the shelves which serve to mix the seed. If the quantity of seed treated at one time is not too large, the stirring of the seed by the rotation of the seed drum is very satisfactory. The efficiency of the machine in mixing the seed and thus assuring uniform heating was tested by embedding a small double-handful of seeds which had been stained red in the middle of the bulk of seeds to be treated and determining the time required for the red seeds to be distributed throughout the larger mass of seeds in the rotating drum. In one test, in which 18 pounds of dry seed (water content 8.5 per cent) were used, the red seeds had become within the lapse of 10 minutes uniformly distributed throughout the entire bulk of seeds in the revolving drum. In another similar test, 27 pounds of seed, a quantity which filled the drum very nearly half full, were used. After three minutes of rotation, the red seeds were present in about equal quantities in the middle region of each of the 6 compartments of the seed drum, and within 45 minutes, they had become distributed fairly uniformly throughout the entire bulk of seeds. Thus, both radial and longitudial mixing was found to be very satisfactory when the seed drum is slightly less than half full. Other tests showed that when the drum is more than half full, mixing is much less satisfactory, because, in a drum of the dimensions used in these tests, the seeds bulk up between the shelves and the axle and fail to slide off the shelves as the drum rotates. If the shelves

were reduced in number from 6 to 4 or 3, the seeds should not so readily bulk between them and the stirring should very probably be satisfactory when the drum is considerably more than half full.

During the course of a treatment, considerable quantities of fuzz wear off the seeds and fall onto the heater shield; this should be cleaned off once or twice during the treatment. In one test in which 3600 grams of seed were treated, 22 grams of fuzz was collected during the treatment.

Certain workers in Egypt, namely, Story[29], [30] and Gough[21] have published descriptions of machines designed to treat cotton seed with dry heat as a means of combatting the pink boll worm. Since the larvæ of this insect are killed within a few minutes by heating infested seeds at temperatures of 60 or 65° continuous operation is possible. By means of belt or screw conveyors, the seeds in contact with hot steam conduits or hot air currents are carried slowly through the heating chamber, traversing the machine and reaching the exit in the course of about 10 minutes. These machines were unknown to the writer until more than two and one-half years after he had first used the one designed by himself for the control of anthracnose, and they are very different from it. Because of the higher temperature and much longer period of treatment required for control of anthracnose than for pink boll worm, it appears that the machines described by Story and Gough are not well adapted to the treatment required to destroy seed-borne elements of the cotton anthracnose fungus.

FIELD, GREENHOUSE AND GERMINATOR TESTS OF SEED TREATED IN BULK

While this investigation has been in progress, seed which had been treated in bulk have been planted in the field, in flats in the greenhouse and in test tubes in order to determine the effectiveness of the treatment in the control of anthracnose.

In 1921, diseased seed which had grown in the season of 1920 and which had been treated in an improvised gas oven as described on page were planted in small plots in fields on the farm of the Negro State Hospital at Goldsboro, N. C., and on the Coastal Plain Branch Station Farm at Willard, N. C. When these plots were visited on August 16, the cotton was in excellent condition, forming a good crop of bolls, but no anthracnose was found at either place. A second and final examination of these plots was made on September 24, 1921. No anthracnose was found on either treated or untreated plots on the farm at the Negro State Hospital. The complete absence of anthracnose from the untreated seed plot may be attributed to the dry weather which prevailed at this place during most of the fruiting season for cotton. At the Willard farm, where more humid conditions had prevailed, 8.6 per cent of the plants on the treated plot and 41.5 per cent of the plants on the untreated seed plot bore one or more bolls infected by the anthracnose fungus. These two plots which were on the same kind of land and had received the same cultural treatment were separated by 10 rows of corn. The results of this test indicate that the treatment given the seed had greatly reduced but had not completely eliminated the disease from the seed.

In 1922, diseased seed which had grown in 1921 and which had been treated in the new seed-treating machine described above were planted on the farms of the two branch stations near Willard and Rocky Mount, N. C. This land had not been in cotton for at least two years. The seed planted near Rocky Mount had been given a preliminary drying at an average temperature of 51° C. for 24 hours and were then heated at the average temperature of 90° C. for 22 hours. The treated seed plot consisted of 15 rows averaging 205 plants each and the untreated seed plot, which was located at a distance of 8 rods from the treated seed plot and separated from it by a corn field, consisted of 19 rows of 120 plants each. The seed planted near Willard had been dried at an average temperature of 50° C. for 24 hours, then heated at an average temperature of 80° for 70 hours. The treated plot consisted of 18 rows averaging 190 plants each and the untreated plot, 40 rods away comprised three rows about 80 rods long. At each farm the treated and untreated seed plots received the same fertilizer application and cultural treatment.

The tests on these two farms were examined twice during August. At the Willard station on August 9, an average of 4 to 5 per cent of the plants on the treated seed plot bore one or more bolls diseased with anthracnose while 17 per cent of the plants on the untreated plot bore one or more affected bolls. On August 30, 30.2 per cent, or 426, of the 1442 plants examined on the treated seed plot bore one or more diseased bolls while 45.7 per cent, or 86 out of 407, plants examined on the untreated seed plot bore diseased bolls. All the bolls on many plants of the check plot were infected while this was not the case on the treated seed plot. Although the treatment given the seed planted at Willard does not appear to have eliminated the disease, it did result in a later appearance and less damage from the disease in the field. At the farm near Rocky Mount, on August 9, only one diseased boll could be found by careful search over all the plants on the treated seed plot while an average of 2 to 3 per cent of the plants on the untreated seed plot bore bolls infected with anthracnose. On August 28, only 20 plants out of 2774 examined, a percentage of 0.7 per cent, on the treated seed plot were diseased; all but four of these were distributed in a small area as if they had become diseased as a result of secondary infections arising from the locus of a single diseased plant. Of the 777 plants examined, 178, or 22.9 per cent, on the untreated seed plot possessed one or more infected bolls.

While it appears that the anthracnose fungus was killed in nearly all the seeds of the treated lot, it also appears that the treatment was not sufficiently severe to completely eradicate the disease from the seed.

In 1923, diseased seed grown in the summer of 1922 was treated and planted at four different locations in the State, namely: on the farm of Mr. J. E. Dougherty, near New Bern, N. C., and on each of the three Branch Experiment Station farms near Willard, Rocky Mount and Statesville, N. C. The cotton on the farm of Mr Dougherty exhibited, on August 14, vigorous vegetative growth, but nearly all the bolls had fallen off because of excessively wet weather and unusual boll weevil activity. No anthracnose was found on either the treated or untreated seed plots. The seed on the farm near Rocky Mount was planted late, which factor combined with

excessively dry weather resulted in poor growth. When examined on September 18, no anthracnose was found on either the treated or untreated seed plots. The seed planted on the farm near Statesville had received treatment consisting of desiccation for 16 hours at 45-50° C. followed by heating for 24 hours at temperatures ranging between a minimum of 80° C. and a maximum of 90° C. When these test plots were examined on October 3, 6 per cent of the plants on the treated seed plot and 5 per cent on the untreated seed plot were found to be diseased. Slightly less than 1 per cent of the plants of the general crop of Early King cotton growing near by were diseased. The seed which were planted near Willard had been desiccated for 20 hours at 45-50° C. and heated for two 10-hour periods on two

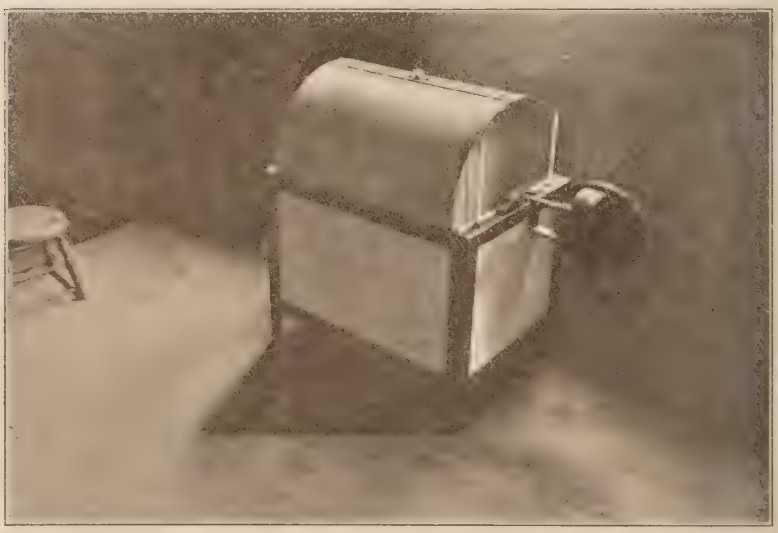


Plate 1. The seed treating machine. Front and right end view showing the speed reducing gears and two thermometers in position.

successive days at temperatures between a minimum of 70° C. and a maximum of 85° C. When these plots were visited on August 15, no anthracnose was found on either the treated or untreated seed plots, although the treated and untreated seed were planted through misunderstanding in adjoining plats. On September 18, 30 per cent of the plants both on the treated and on the untreated plots bore one or more infected bolls. Since anthracnose lesions appeared to have originated at punctures made by boll weevils, these insects were suspected of being responsible for this distribution of disease in both plots. Accordingly, a large number of them (99 in all) were collected in sterile tubes and carried back to the laboratory. Each weevil was shaken in 5cc. of water which was in turn poured into a plate of sterile nutrient agar. Colonies of the anthracnose developed in 17 per cent of the plates and in about one-third of these, anthracnose was the dominating flora. It is highly probable, therefore,

that failure to find any difference in the amount of anthracnose on the treated and untreated plots in this test was due to the spread of spores of the anthracnose fungus from diseased to healthy plants by this insect.

A check on the results of the field trials on the farms near Statesville and Rocky Mount were made by plantings in the greenhouse. Four hundred seeds of each treated lot and 200 untreated seeds were chosen for these tests. By the end of 8 days, the germination was 92 and 96 per cent respectively, for the treated and 92 per cent for the untreated seed. By the seventeenth day, the percentage of anthracnose was 2 and 6 per cent respectively for the treated seed and 45 per cent for the untreated. The evidence from field and greenhouse trials is, therefore, in accord in showing that dry

PLATE 2. The seed treating machine. Front and left end view with hood removed and door open. The seed drum and the shield over the heaters are shown in position. The position of the thermostat which is here located inside the upper left corner, has been changed to the upper part of the front wall where it is more directly exposed to the hot air current which heats the seeds. The small condenser shown on the floor is connected across the points of the thermostat in order to minimize arcing and consequent clatter of the circuit breaker.

heat treatment greatly reduced the amount of viable anthracnose fungus present in these seed without impairing germination, but that the treatment was not sufficiently severe to completely destroy the fungus.

In still another test with seed of the 1922 crop, a quantity of seed was heated in the seed-treating machine at a temperature of 50° for 24 hours, then 100° C. for 12 hours. One thousand of these treated seed were planted in sand in flats in the greenhouse. Only 3 per cent of these seed germinated in 17 days making it evident that a temperature of 50° does not accomplish sufficient desiccation to enable the seed to withstand 100° for 12 hours. This result is in accord with results of tests on small lots of seed in the electric oven.

In 1924, highly infected seed which had grown in 1923 were treated. Inasmuch as the temperatures applied in previous years had failed to eradicate the disease and still leave the seed in a condition for good germination, higher temperatures were employed in the 1924 tests. In these tests, the seed were dried at 60-65° C. for 22 hours and heated at 95-100° C. for 12 hours. Because of the fact that the boll weevil now present in great numbers is known to carry spores of the cotton anthracnose fungus after visiting diseased bolls and may in this way transport the spores from diseased to healthy cotton over long distances; none of the seed treated in 1924 were planted in the field, but the efficiency of the treatment was tested by growing seedlings in sand in the greenhouse and in test tubes. Flats of sandy soil in the greenhouse were sterilized by drenching with formaldehyde and closely covering with paper for 2 days. The paper was then removed and the formaldehyde was allowed to evaporate over a period of 3 weeks. Three different tests of the lot of seed treated as described above were made. In test 1, 500 treated and 300 untreated seeds, and in test 2, 500 treated and 400 untreated seeds were planted in the flats. In test 3, 100 treated and 50 untreated seeds were germinated in test tubes. Test 1 was planted on June 16, test 2 on July 7, and test 3 on July 11. The seedlings in the greenhouse were kept well watered in order to encourage development of the disease on any of the seedlings which might be infected. Beginning on the eighth day and continuing at intervals of two days, all the seedlings from one or more of the flats were pulled. If lesions were present on cotyledons or stems, they were examined microscopically to determine whether they were caused by the anthracnose fungus.

TABLE XIV

Per Cent of Anthracnose and Germination in Cotton Seeds Treated in the Seed Treating Machine. The Seeds Were Dried at 60-65° C. for 22 Hours, Then Heated at 95-100° C. for 12 Hours. Cook Cotton of 1923.

Test No.	How Tested	Per Cent of Anthracnose		Per Cent Germination		Number of Seeds Planted	
		Treated	Untreated	Treated	Untreated	Treated	Untreated
1	In flats in greenhouse	0	14.8%	92.4%	92.0%	500	300
2	In flats in greenhouse	0	2.6%	92.0%	96.7%	500	400
3	In test tubes	0	26.0%	99.0%	98.0%	100	50

Table XIV presents the summarized results of each of these tests. No anthracnose developed on any seedling which came from treated seed while as many as 26 per cent (test 3) of the seedlings growing from untreated seed became diseased. The percentage of germination of the treated seed exceeded that of the untreated in tests 1 and 3 and was slighly less than that of the check in test 2.

GENERAL CONCLUSIONS

Consideration of the experiments described above leads directly to the conclusion that the practical and complete control of cotton anthracnose in its seed-borne aspects can be obtained by the use of dry heat. The water content of the seeds which are to be heated is a factor of primary importance in determining the essential details of treatment. Control cannot be accomplished by the use of seed containing an amount of moisture normal to ordinary storage conditions, for dry heat at temperatures effective in killing the anthracnose fungus carried by such seed either completely destroys is viability or seriously reduces its germination. Air dry seed subjected to dry heat temperatures of 80, 90, 95 and 100° C. succumb before the anthracnose fungus is killed and at temperatures of 70° or less the vitality of the anthracnose fungus in not destroyed. However, after the moisture content of cotton seed has been sufficiently reduced by use of relatively low temperatures for appropriate periods of time, they may safely be treated at temperatures sufficiently high to prevent subsequent development of anthracnose on the seedlings. The vitality of the spores and mycelium of the anthracnose fungus carried on diseased cotton seed can be destroyed by the application of dry heat at temperatures of 95° C. for 12 hours or 100° C. for 6 hours. Temperatures of 80° for 72 hours, and 90° for 24 hours, accomplish the same result in all but a fraction of 1 per cent of the seeds, and it appears that prolonging the treatment at 80° and 90° to 96 and 48 hours respectively should destroy the seed-borne fungus in all the seeds. When cotton seed have been predried at proper temperatures for suitable periods of time, the application of dry heat at 80° for 72 hours, 90° for 24 hours or 95° for 12 hours, causes little or no reduction in percentage of germination. 100° C. for 6 hours causes a very considerable diminution in percentage of germination; however, the number of seeds which survive the treatment is sufficiently great to provide for an ample stand in the field.

The anthracnose fungus carried by a small proportion of the seeds is much more resistant than that carried by the majority of the seeds. Although 14 hours of heating at 90° reduced the anthracnose to 2 per cent, the fungus still survived in a small percentage of the seeds in 2 out of 8 tests in which heating continued for 18 hours and in 1 out of 9 tests in which heating lasted 24 hours. It may be that in the few seeds in which the fungus survived the relatively long periods of heating, the hyphæ of the fungus had penetrated deeply within the embryo of the seed and its protoplasm was by reason of its position embedded within other living tissue protected from the extreme desiccating and coagulating effects of the heat.

Dry heat treatment of cotton seed has certain obvious advantages over any method previously described for control of seed-borne anthracnose. It destroys all of the anthracnose fungus carried by diseased seed while chemical disinfectants and corrosives are only partially effective at best. It may be used in a much wider range of effective temperatures (80 to 100° C.) than is possible with the hot water method, and the period of treatment at any temperature within the effective range may be greatly

prolonged without danger of reducing germination of the seed. It is an effective means by which the loss of time occasioned by the ageing of seed may be avoided; for diseased seed which has been properly treated by dry heat may safely be planted the season immediately following its production. Moreover, the seed grower need not assiduously avoid selection of seed from disease-free plants. The dry heat treatment for cotton anthracnose described by Lipscomb and Corley not only requires the reduction of the water content of the seed by strong preliminary desiccation before the application of the high temperature effecting control of the disease but also specifies that the seed be heated in a vacuum or in an inert gas, as nitrogen. The dry heat method described in this paper has the advantage of being less complicated in operation. The use of a vacuum or inert gas is not required, the seeds being heated in air after suitable preliminary desiccation.

By the use of a machine equipped with a proper stirring device, cotton seed may be successfully treated in bulk with dry heat. In the operation of the machine described in this paper, uniform heating is accomplished because of very thorough mixing of the seed, and for this reason, this type of machine is believed to be well adapted to the treatment of cotton seed with dry air at high temperatures. With this machine, the effective treatment for seed in bulk for control of the anthracnose disease without serious diminution of germinability consists of 20 to 24 hours of desiccation at 60 to 65° C., followed by 12 hours of heating at 95-100° C.

Practical application of the method of treatment herein described may be made by seed companies or associations interested in the production and sale of anthracnose-free seed, or by ginners who could treat seed for their patrons at a fixed price per bushel. By use of a machine of greater capacity than the one used in these tests, much larger quantities of seed can be treated at one time. A reliable source of heat is the prerequisite to successful operation. In large machines, steam heat could readily be substituted for electricity and the treatment could be made continuous by so constructing the machine that the hot air which has been used to heat the seed in the second stage of the treatment can be used to dry seed of a second and perhaps even a third lot which is at the same time passing through the first or drying stage, in which the aim is merely to reduce the water content of the seeds.

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II. GERMINATION OF COTTON SEED AND VIABILITY OF ANTHRACNOSE AS INFLUENCED BY DESICCATION AND BY STORAGE IN INERT GASES

The foregoing paper has been devoted to a method of treating cotton seed with dry heat for the purpose of destroying spores and mycelium of the anthracnose fungus. It is the purpose of this paper to describe certain other experiments which were conducted in order to study the germinability of cotton seeds which have been subjected to various treatments and to show the correlation between the water content and the resistance of cotton seeds to high temperatures and anthracnose control.

HISTORICAL

As early as 1834 Edward and Colin[21] tested the effect of heat on the vitality of the seeds of wheat, barley, rye, bean and flax. When heated in water for 15 minutes, all the seeds were killed by a temperature of 75° C. and the upper temperature limit at which the seeds were not injuriously affected was found to be 50° C. When the seeds were heated in water

vapor, all were killed at 75° C., but at 62° C. little harm was done, whereas when heated for 15 minutes in dry air at 75° C. many were capable of germination. It was thus found that the medium in which the seeds were heated determined the temperatures that they were able to endure without loss of germinative power.

In 1879, Noble[44] heated wheat, rye, and oats seeds having a water content had been heated for one hour in water at 55° C. and that all were killed when heated at 60° C. When heated in dry air, the seeds endured temperatures of 65° C. for 4 hours, 85° C. for 30 minutes, and 90° C. for 15 minutes, but all were killed in 15 minutes by a temperature of 95° C., and in 19 minutes by a temperature of 100° C.

In 1863, Haberlandt[29] heated 88 species of dry seeds in dry air at 100° C. for 48 hours. Twelve species entirely lost their power to germinate, 12 species suffered partial killing, and 64 species gave full germination, many of this last group, however, showing marked retardation. When the same kinds of seeds were heated at 87.5° C. for 48 hours, only 2 species completely lost their power to germinate, 17 species were partially killed, 34 species showed marked retardation of germination, and 35 species manifested an acceleration of germination. No injury was observed to result from an application of a temperature of 56-57° for 48 hours.

Haberlandt[29] also studied the effect of inbibed water on the viability of seeds of rye, corn, flax and peas. Air dried seed and seeds which had been allowed to swell in water at a temperature of 12 to 15° C. for 24 hours were heated in water at a temperature of 30-55° C. for periods of 5-10 hours. With every species, the soaked seeds proved the more susceptible to the injurious effects of heat, as evidenced by the greater reduction of total percentage of germination and more pronounced retardation in rate of germination.

In 1897, Noble[44] heated wheat rye, and oat seeds having a water content of 12 to 13 per cent at temperatures of 40 to 100° C. in dry air. Wheat and rye suffered no destruction of vitality when heated at 80° C. for a period of time sufficiently long to reduce the water content to 1 or 2 per cent. Heating at 90° C. for 2 days, whereby the water content was reduced to less than 1 per cent, first lessened the power of germination, and a temperature of 100° C. for 24 hours almost completely destroyed it. Oats was reported to be more susceptible to high temperatures than wheat or rye. Drying oats for 3 days at 50° and 60° strongly retarded and delayed germination.

In 1899, Jodin[28] showed that the water content of seeds profoundly influences the time which they can survive high temperatures. This worker heated wheat seed in a vacuum to a temperature of 100° C. without destroying its power to germinate. Pea and cress seeds were killed when heated at 98° C. for 6 hours, but when seed from the same lots were heated at 60° C. for 24 hours, then at 98° C. 30 per cent of the peas and 60 per cent of the cress still germinated. When seeds were heated in sealed tubes at 40° C., they were killed in 20 days, but, when the seeds were sealed up with CaO, germination was not reduced by heating for 206 days at 40° C.

Dixon [30, 45] in 1901 and 1903 found that seeds of many species which had been dried over H_2SO_4 or in an oven which was slowly raised to a temperature of 90° could thereafter endure, without complete loss of ability

to germinate, temperatures ranging from 100 to 121° C. for a period of one hour. He found that *Avena sativa* and *Hordeum distichum* withstood a temperature of 118° C. and *Medicago sativa* survived 121° C. although exposure at temperatures near the maximum increased the time needed for germination.

In 1917, Wagonner[4] published the results of experiments on the effect of different temperatures on the viability of radish seeds whose water content varied from 0.4 per cent to 71 per cent. The temperatures employed ranged from 50 to 125° C. and the seed were heated in stoppered flasks, immersed in a water bath. When heated at 60° C., those seeds whose initial

PLATE 4. Cleveland big boll cotton of the 1924 crop growing in the field after a dry heat treatment which consisted of preliminary desiccation at 60-65° for 22 hours and subsequent heating at 95-100° C. for 12 hours. This seed was of poor quality to begin with because of unfavorable weather at the time it was maturing, and the treatment reduced the germination from 69 per cent to 60 per cent. The seed was planted on April 23 and the photograph was taken on May 21. During most of this time, the weather was very unfavorable for growth of cotton. From 50 to 75 per cent of the seedlings of the general crop died largely from an attack of anthracnose following cold weather and the greater portion of the acreage had to be replanted. In the treated plot sickly seedlings were extremely rare and in no case could anthracnose spores be found on the few which were present while anthracnose spores were abundant on 90 to 100 per cent of the sickly seedlings found on untreated plots. Although the seedlings on the rteated plots were making but little growth they were sturdy, remarkably healthy and possessed strikingly good color in comparison with seedings from untreated seed on nearby plots.

water content was 45 per cent or more were killed whereas those containing only 4 per cent of water suffered no reduction in percentage of germination. Complete loss of viability occurred when seeds containing 4 per cent of water were heated at 100° C. while on the contrary seeds containing only 0.4 per cent of moisture gave as high a percentage of germination as untreated seed. A small percentage of the seeds containing 0.4 per cent water survived a temperature of 123° C. Thus the resistance of radish seeds

to heat was found to bear an inverse relation to initial water content at the time of heating. Temperatures approaching the maximum for the seed of a given water content caused more or less pronounced retardation of germination.

Burgess[6] in 1919 published data showing the percentage of germination of certain agricultural seeds which had been subjected in an air dry condition to high temperatures. Wheat and rye were killed by an exposure of one hour to 248° F. but gave respectively 55 per cent and 78 per cent germination when heated for 3 hours at 230° F. Ninety-eight per cent of oats and 2 per cent of cowpea seeds germinated after 5 hours of heating at 194° F. Soybeans gave perfect germination after exposure for one hour at 194° F., and soybeans, garden beans and cowpea seeds gave respectively 100 per cent, 92 per cent and 78 per cent germination after subjection for 3 hours to heat at 176° F.

In 1920, Saillard[47] subjected sugarbeet seed of three grades designated "very good," "good" and "poor," which gave before treatment, 98, 87 and 36 per cent germination respectively, to dry heat at temperatures ranging from 60 to 105° C. After treatment at 80° C. the respective percentages of germination for the three lots was 97, 86, and 9 per cent; after treatment at 90° C., 95, 75 and 2; and after treatment at 100° C., 80, 46 and 0. Of the "very good" seeds 3 per cent survived the treatment at 105° C.

In 1922, Gain[22] published the results of experiments with sunflower seeds. He found that by carefully drying and heating the seeds for suitable periods of time at temperatures which were raised by successive steps, many of the seeds could be prepared to endure temperatures as high as 150° C. After similar treatment, seeds of a number of other plants were found to endure temperatures of 110 to 155° C. without complete loss of power to germinate. Similar experiments by Sigalas and Marneffe[51] confirm the work of Gain.

In addition to the workers whose papers are reviewed in the above paragraphs, others as Groves,[28] Crocker and Groves,[10] Goodspeed,[27] Harrington and Crocker,[31] Honing,[35] Hausdorfer,[32] Schneider-Orelli,[49] Neuberger,[45] Just,[39] and Schubert[50] have published from time to time the results of studies on the effect of desiccation and the application of heat on the viability, longevity and germination of seeds. In general, these workers have found that air dry seeds are much more resistant to dry than to moist heat and that preliminary desiccation over chemicals or at low temperatures greatly increases the ability of seeds to withstand exposure to high temperatures.

Few workers have published any data showing the effect of dry heat on cotton seed. The publications of Story,[53, 54] Gough[24] and Lipscomb and Corley[43] have been cited in an earlier part of this paper. Gough, using a machine designed for treatment of cotton seed to kill the pink boll worm found that, when the seed remained in the machine for nine minutes and the temperature of the seed did not exceed 51° C., very little reduction of germination resulted, but injury was marked at 61° C. and very severe at 72° C. Lipscomb and Corley found that ,while cotton seeds in an air

dry condition are quickly destroyed by a temperature of 100° C., they may endure this temperature for 26 hours without loss of viability if they are first thoroughly dried and then heated in a vacuum or in an inert atmosphere as nitrogen.

EXPERIMENTAL

A number of experiments were performed in order to determine the temperature and period of time most suitable and practical for drying cotton seed in order to condition them to withstand the higher temperatures required for killing the anthracnose fungus. In certain of these experiments the extent of dehydration was measured by determining the proportion of water lost during the treatment. In all these tests, precaution was taken to have the separate lots reasonably uniform by first selecting and thoroughly mixing enough seeds for the entire experiment then mechanically dividing the heap into the number of lots required. Each lot was dried separately in one electric oven and heated in another, so it was not necessary to change the adjustment of the oven thermostat during the course of an experiment.

Table I gives the data of Experiment 1 in which 5 lots of seed were dried at temperatures of 40, 45, 50, 55 and 60° C. for 24 hours and afterward heated at 90° for 18 hours. The percentage of germination was recorded at the end of 2, 3, 4, 5, 6, 9 and 14 days.

TABLE I

EFFECT OF DESICCATION AT TEMPERATURES OF 40, 45, 50, 55, AND 60° C. FOR 24 HOURS AS A MEANS OF PREPARING COTTON SEED TO WITHSTAND A TEMPERATURE OF 90° C. FOR 18 HOURS. 50 SEEDS IN EACH LOT. CROP OF 1922.

Exp. No.	Lot No.	Preliminary Drying		Subsequent Heating		Per Cent of Germination						
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Days in Germinator						
						2	3	4	5	6	9	14
1	1	40	24	90	18	0	4	16	22	28	28	36
	2	45	24	90	18	0	28	60	62	62	62	62
	3	50	24	90	18	4	74	92	....	96	96	96
	4	55	24	90	18	2	76	96	....	96	96	96
	5	60	24	90	18	2	66	96	....	96	98	98

Lots 1 and 2, which had been dried at 40 and 45° C. respectively showed marked diminution in both the rate and final percentage of germination. Temperatures of 40 and 45° C., when applied for periods not longer than 24 hours, do not condition cotton seed to endure without injury to germination the temperature of 90° C. for 18 hours. For this purpose it is necessary to use a temperature of 50° C. or above. In all lots which were dried at 50° C. or above, germination was prompt and entirely satisfactory in amount.

In Experiment 2, the results of which are set forth in Table II, the temperature of drying was kept the same for all lots but the period of heating was varied. Four lots were dried at 50° C. for 12, 18, 24 and 36 hours, and subsequently heated at 90° C. for 24 hours. The percentage of germination

was recorded at the end of 7, 13 and 19 days and the radicles of all the seedlings which had germinated and remained healthy were measured at the end of seven days. From these measurements the average length of the seedlings in each lot was determined and recorded, and the measurements are arranged in groups on the basis of the length of the seedlings and recorded in the table in order to show the correlation which exists between the period of drying and the rate of germination.

The percentage of germination of the lots treated for 12, 18, 24, and 36 hours, was 62, 92, 96, and 96 respectively on the 19th day, and the average length of the radicles was 20.6, 46.7, 44.6, and 51.7 mm. respectively on the 7th day. The correlation table shows that germination of lot 1 was not only greatly reduced in amount but also markedly diminished in rate, for the large groups of seedlings in this lot are to the left of the table and in the classes characterized by short radicles; while in lots 2, 3, and 4, the large groups are at the right side of the table and in classes characterized by long radicles. The facts of the reduced amount and retarded rate of germination and the short average length of the seedlings in lot 1 point very definitely to the conclusion that when seeds are to be heated at 90° for 24 hours, the 12-hour period of drying at 50° C. is too short. The 18-hour period is much better and the 24 and 36 hour periods are entirely satisfactory.

Experiment 3, the results of which are also presented in table II, is like experiment 2 in every detail except that after preliminary drying at 50° C. the seeds were heated at 95° C. for 8 hours. In this experiment, the germination of the 4 lots dried for 12, 18, 24 and 36 hours, was 38, 68, 90, and 96 per cent respectively and the average length of the radicles of the seedlings increased with increase in length of the period of drying. In lots 1, 2, and 3, germination was both reduced in amount and retarded in rate and only in lot 4, which was dried for 36 hours, did germination occur without evidence of injury. It is evident from the results obtained from this experiment that when seeds are to be heated at 95° C. for as long as 8 hours, the 12, 18 and 24-hour periods of drying at 50° C. do not give sufficient protection while the 36-hour period affords ample defense against the ordinarily injurious effects of the higher temperature.

Since in experiments 1, 2, and 3 described above the protection given the seeds by the preliminary drying increased within limits with increase in both temperature and time of drying, it seemed reasonable to assume that the increased protection is due primarily to the loss of water which occurs during the period of preliminary drying. In order to test this matter, certain experiments were made in which the amount of water lost was determined by weighing the seeds accurately at different stages in the course of the treatment. The water loss during the period of the preliminary drying and during the time of heating at 95° C. was determined for each lot, and is given in Table III as percentage of the original air dry weight of the seeds. The total water content of a lot of seeds comparable in every way to the treated lots was determined by heating 100 seeds to constant weight at a temperature of 103-105° C., and this value is recorded in the table also as percentage of the air dry weight of the seeds. Using the values obtained for the water content of the air dry seeds and for the

TABLE 11

EFFECT OF DRYING AT 50° C. FOR DIFFERENT PERIODS IN CONDITIONING COTTON SEED TO WITHSTAND TEMPERATURES OF 90 AND 95° C. 50 SEEDS IN EACH LOT. COOK COTTON OF 1923 CROP.

Exp. No.	Lot No.	Preliminary Drying		Heating		Per Cent Germination			Correlation of Period of Preliminary Drying With Effectiveness thereof*									
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Days 7	13	19	Group Characteristics, Length and Number of Seedlings in Each 0–9 mm.	10–19 mm.	20–29 mm.	30–39 mm.	40–49 mm.	50–59 mm.	60–69 mm.	70–79 mm.	80–89 mm.	Average Length of Seedlings mm.	Number Seedlings Measured
2	1	50	12	90	24	50	58	62	9	6	2	3	2	2	1	0	0	20.6	25
	2	50	18	90	24	92	92	92	5	2	4	3	7	10	10	4	1	46.7	46
	3	50	24	90	24	96	96	96	3	3	5	3	12	10	9	3	0	44.6	48
	4	50	36	90	24	96	96	96	2	2	3	8	4	7	10	8	4	51.7	48
3	1	50	12	95	8	28	38	38	4	3	5	0	0	0	1	0	0	18.0	13
	2	50	18	95	8	64	66	68	10	3	4	4	5	3	0	0	1	25.6	30
	3	50	24	95	8	74	80	80	10	4	5	1	8	6	1	1	1	31.0	37
	4	50	36	95	8†	96	96	96	0	1	2	4	14	14	8	4	0	50.4	48

*As indicated by the average length of the seedling radicles and by the number of seedlings in the different growth groups characterized by length at the end of 7 days in the germinator.

†This lot actually ran 9½ hours at 95° C.

water lost by the air dry seeds during the period of preliminary drying, the water remaining in the seeds at the end of the period of preliminary drying was calculated and is given in the table as percentage of the oven dry weight of the seeds. In Experiment 4 the preliminary drying was done at 60° C. for periods of 12, 18, and 24 hours, and in experiment 5 at 50° C. for 24 and 36 hours. In experiment 6 the different lots were dried at 40, 45, 50, 55, and 60° C. for a uniform period of 24 hours. In all three experiments the final treatment was done at 95° C. for 12 hours. After the heat treatments the seeds were germinated between blotters and the percentage of seeds which sprouted was recorded at intervals in order to get some idea of the rate of germination. Also the average length of the radicles of the seedlings which were not attacked by bacteria or fungi was determined at the end of the 5-day period in the germinator.

All the seeds dried at 40° C. and all except 5 dried at 45° C. were killed by the subsequent treatment at 95°. During the preliminary period of drying these seeds lost water equivalent to 3.5 per cent or less of their air dry weight and retained an amount of water equal to 5.54 per cent or more of their oven dry weight. The two lots which were desiccated at 50° for 24 hours and the two lots dried at 60° for 12 hours were retarded in rate and greatly reduced in amount of germination. These seeds lost more water during the period of preliminary drying than those which were dried at 40 and 45°, but the amount retained was still too great to enable the seeds to withstand well the treatment at 95°. The seeds which were dried at 60° for 18 and 24 hours suffered no ill effects from the treatment at 95°, but on the contrary germination was as complete and somewhat more prompt than in untreated check seeds. During the desiccation at 60° these seeds lost water equal to 5.24 per cent or more of their air dry weight and retained an amount equivalent to 3.36 per cent or less of their oven dry weight. In no test in which the final treatment of the seeds was at 95° for 12 hours and in which the seeds lost as much as 5.24 per cent of their weight during the period of preliminary drying did the treatment at 95° cause pronounced loss of viability. The lot which was dried at 55° and which lost 5.51 per cent of its weight appears to have suffered slight reduction in amount of germination. Aside from this one lot, the seeds which lost as much as 5.24 per cent of their weight germinated more promptly and as completely as untreated seed. As a result of the study made in experiments 4, 5, and 6, a statement expressing the relation between water loss and resistance to high temperature may be formulated to the effect that cotton seeds may be conditioned to withstand a temperature of 95° C. for 12 hours by desiccating them at low temperatures until they have lost not less than 5.24 per cent of their dry weight. Obviously, this statement can apply only to seeds whose water content is not much greater than 8.33 per cent or 8.57 per cent of their air dry weight; for with seeds containing more than this amount of water, the removal of 5.24 per cent of their weight would still leave the living protoplasm too strongly hydrated to long withstand a temperature of 95°, while, on the other hand, seeds containing less than 8.33 per cent of water can obviously be conditioned to withstand 95° for 12 hours by removal of water equivalent to less than 5.24 per cent of their dry weight. A much more broadly applicable criterion of the ability of seeds

TABLE III

WATER LOSS AND WATER CONTENT AS RELATED TO DIFFERENT PERIODS OF PRELIMINARY DESICCATION AND TO GERMINATION OF COTTON SEEDS SUBSEQUENTLY HEATED AT 95° C. FOR 12 HOURS. 100 SEEDS IN EACH LOT. COOK COTTON OF 1923 CROP.

Exp. No.	Lot No.	Preliminary Treatment		Final Treatment		Per Cent Germination in Germinator						Average Length of Radicle at end of 5 days mm.	Number Seedlings Measured	Water Content in Per Cent †	Per Cent Water Loss by Prelim. Treatment †	Per Cent Water in Seeds After Prelim. Drying *
		Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	68 Hours	72 Hours	91 Hours	5 Days	7 Days	9 Days					
4	1	60	12	95	12	7	.....	53	66	70	71	9.5	51	8.33	4.58	4.11
	2	60	18	95	12	58	.....	96	96	96	96	.....	.....	8.33	5.38	3.19
	3	60	24	95	12	66	.....	97	97	97	97	16.4	96	8.33	5.68	2.88
	4	Check	Untreated			32	.....	85	91	96	96	12.6	85	8.33	.....	.....
	5	60	12	95	12	25	.....	76	80	81	82	.....	.....	8.33	4.88	3.74
	6	60	18	95	12	68	.....	98	98	98	98	.....	.....	8.33	5.24	3.36
	7	60	24	95	12	52	.....	95	95	95	95	.....	.....	8.33	5.60	3.00
5	1	50	36	95	12	43	.....	87	92	93	93	13.3	86	8.33	5.01	3.62
	2	50	24	95	12	14	.....	47	61	66	66	.....	.....	8.33	4.55	4.11
6	1	40	24	95	12	0	0	0	0	0	.....	.....	.....	8.57	2.68	6.43
	2	45	24	95	12	0	0	1	2	5	.....	.....	.....	8.57	3.50	5.54
	3	50	24	95	12	4	21	46	56	64	.....	12.6	48	8.57	4.99	3.90
	4	55	24	95	12	13	53	83	89	90	.....	13.9	78	8.57	5.51	3.34
	5	60	24	95	12	16	59	96	99	99	.....	15.5	94	8.57	6.08	2.72
	6	Check	Not treated			27	47	88	92	94	.....	15.6	91	8.57	.....	.....

†Based on original air dry weight of seeds.
*Based on oven dry weight of seeds.

to withstand the temperature of 95° for 12 hours is found in the amount of water contained by the seed when this is expressed as a ratio or as a percentage of the oven dry weight of the seeds. The oven dry weight of the seeds furnishes a nonvariable base for computation of the ratios or percentages expressing the moisture content. Without exception, when the moisture content after the preliminary drying was as great as 3.9 per cent of the oven dry weight, the viability of the seeds was seriously impaired by heating at 95° for 12 hours.

When the moisture content after the preliminary drying was not greater than 3.62 per cent of the dry weight of the seeds, no serious loss of viability resulted from the subsequent treatment at 95°. Furthermore, when the water content after drying was not greater than 3.19 per cent of their dry weight, the seeds heated at 95° for 12 hours germinated more promptly than the untreated seeds used for a check. The statement that, when the moisture content of cotton seed is not greater than 3.19 per cent of their dry weight, the seed may be safely subjected to a temperature of 95° for 12 hours without consequent loss of vaibility may then be considered to apply to any lot of normal cotton seed.

In the course of these studies certain other experiments were performed which further show the relation of time and temperature as factors influencing the water content of seeds during desiccation. In one of these experiments, cotton seeds were desiccated in a gas oven at a temperature of about 45° C., and at intervals of 24 hours the seeds were cooled in a desiccator, carefully weighed in order to determine loss of water and returned again to the oven. At the end of 144 hours, the temperature was raised to 60° C. and weighings were again made at the end of 24 and 48 hours. In another test seeds being dried at 60° C. in an electric oven were weighed at frequent intervals until their weight became practically constant. The temperature was then raised to 70° C. and the seeds were weighed after periods of 24, 48, 72, and 96 hours, and finally heated at 103° C. in order to determine their total water content. The results of these tests were presented in Table IV.

In experiment 7 the water content of the seeds rapidly diminished during the first 24 hour period of drying and continued to decrease markedly, but less rapidly, during the second and third periods. By the end of 72 hours, the weight of the seeds had became practically constant, only insignificant amounts of water, cumulatively less than 0.5 per cent, being lost during the three succeeding 24-hour intervals. When at the end of 144 hours the temperature was raised to 60° C., a marked reduction of water content of the seeds again occurred by the end of 24 hours. Little water was lost during the second 24-hour period of heating at 60°. By this time, the weight of the seeds and their water content had become nearly constant for the temperature of 60° C.

TABLE IV

SHOWING THE RELATION BETWEEN TIME AND TEMPERATURE AND LOSS OF WATER BY GIVEN LOTS OF SEED IN DRY AIR.

Exp. No.	Preliminary Desiccation		Subsequent Desiccation		Weight (Water) Lost		Increase in Per Cent of Water Lost
	Temp. Deg. C.	Time Hours	Temp. Deg. C.	Time Hours	Grams	Per Cent of Wet Weight	
7*	45	24			0.211	4.16	
	45	48			0.242	4.77	.61
	45	72			0.270	5.33	.56
	45	96			0.275	5.42	.09
	45	120			0.279	5.50	.08
	45	144			0.289	5.69	.19
	45	144	60	24	0.360	7.10	1.41
	45	144	60	48	0.374	7.38	.28
8†	60	14			1.133	9.91	
	60	23			1.185	10.37	.46
	60	40			1.216	10.64	.27
	60	62			1.234	10.80	.16
	60	86			1.237	10.82	.02
	60	86	70	24	1.292	11.31	.49
	60	86	70	48	1.322	11.57	.26
	60	86	70	72	1.329	11.63	.06
	60	86	70	96	1.32		

*50 seeds, water content 10.57 per cent of wet weight, heated in gas oven.
†100 seeds, water content 13.20 per cent of wet weight, heated in electric oven.

In Experiment 8, in which seeds were heated directly at 60° C., weight and water content of the seeds rapidly decreased during the first 14 hours and continued to decrease, although at a diminishing rate, until it became constant at the end of 62 hours. When the temperature was raised to 70°, a marked decrease of weight again occurred during the succeeding period of 24 hours, and the water content again became practically constant by the end of 48 hours.

The results of these experiments are graphically illustrated in Fig. 1. The curves show the relation of time to loss of water from seeds which were being subjected to two different desiccatory dry heat treatments. Both curves rise rapidly at first and then flatten out indicating that longer desiccation at that particular temperature does not cause further dehydration. Later, the increase in the desiccatory temperature of the treatments is marked by an abrupt rise in each curve, and this is followed again by a flattening of the curves as desiccation once more approaches its maximum for the particular temperatures being used. If the temperature is too low, the time of desiccation may be prolonged indefinitely without attaining the degree of desiccation requisite to enable cotton seeds to withstand without loss of viability the higher temperatures which must be used to free it from anthracnose. On the other hand, when an adequate temperature is used, a more or less definite minimum of time is necessary in order to accomplish a degree of desiccation sufficiently pronounced to protect the seeds.

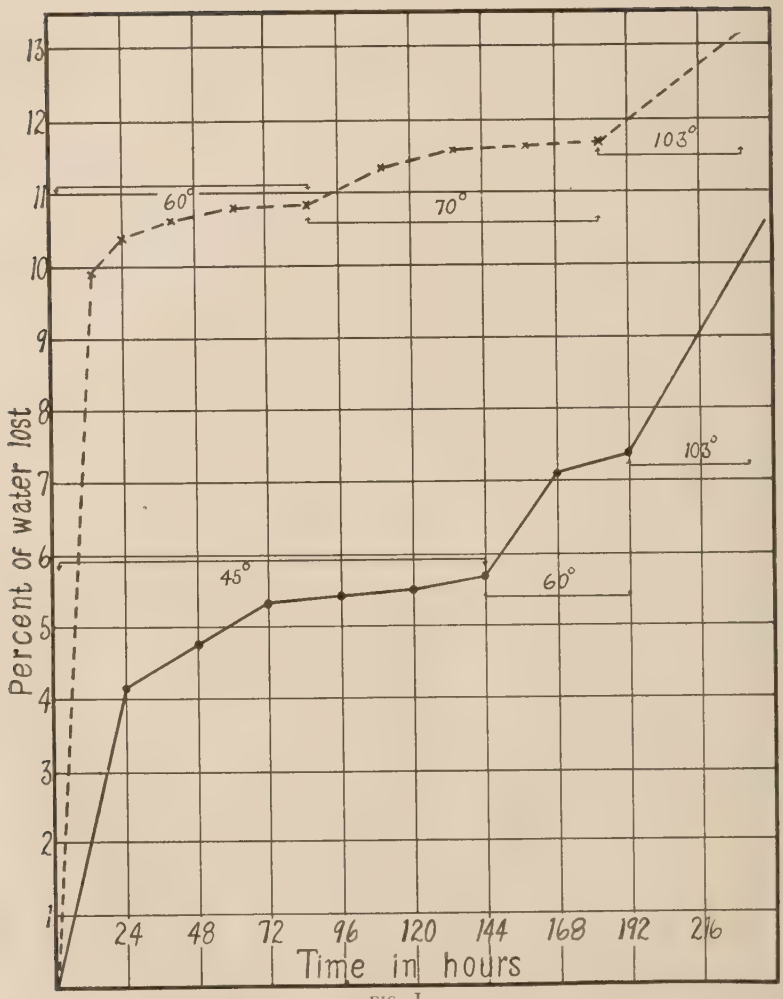

FIG. I

FIG. I. Curves which are based on the data presented in table IV and which show the relation between temperature and time of desiccation to loss of water by two lots of seed during desiccation. The curves rise rapidly at first but, after a more or less definite period of heating at a given temperature, each curve assumes a horizontal position denoting equilibrium between the drying power of the air and the forces by means of which the seed retains water. When the temperature of drying is increased, each curve rises abruptly again and, after a time, assumes a horizontal course once more, indicating another point of equilibrium. Unless the temperature of drying is sufficiently high, seeds cannot be reduced to the state of desiccation which will enable them to withstand the high temperatures required to kill the seed-borne anthracnose fungus although the period of heating be greatly prolonged. The lot of seeds represented by the lower curve was heated in a gas oven, and those represented by the upper curve, in an electric oven.

TABLE V

ACCELERATED GERMINATION OF COTTON SEED FOLLOWING THE TREATMENT TO DESTROY ANTHRACNOSE. 100 SEEDS IN EACH LOT.

Exp. No.	Lot No.	Preliminary Drying		Heating		Seed Coats	Per Cent of Germination at end of Time Indicated					Average Length of Radicle in mm.		Number of Seedlings Measured	
		Temp. Deg. C.	Time Hours	Temp. Deg C.	Time Hours		26 Hours	45 Hours	57 Hours	76 Hours	91 Hours	76 Hours	91 Hours		
1	1	60	18	95	12	Removed	62	88	88	93		26.3		54	
	2	Not Dried		Not Heated		Removed	91	99	99			38.3		62	
	3	60	18	95	12	Intact	1	23	87	93	95		24.3	79	
	4	Not Dried		Not Heated		Intact	0	18	65	87	91		21.2	80	
							24 hrs.	47 hrs.	64 hrs.	112 hrs.		72 hrs.	112 hrs.		
2	1	60-65	22	95-100	12	Removed	38	96				17.9	32.5	88	
	2	Not Dried		Not Heated		Removed	75	100				23.2	36.0	97	
	3	60-65	22	95-100	12	Intact	0	31	89	93			23.2	78	
	4	Not Dried		Not Heated		Intact	0	18	85	96			24.8	88	
							46 hrs.	56 hrs.	78 hrs.	168 hrs.					
3	1	60-65	22	95-100	12	Intact	57	82	86	86					
	2	Not Dried		Not Heated		Intact	40	80	90	91					
							46 hrs.	52 hrs.	71 hrs.						
4*	1	60-65	22	95-100	12	Intact	43	69	93						
	2	Not Dried		Not Heated		Intact	42	68	92						

*This Experiment differs from Exp. 9 only in that the blotters were permitted to become somewhat dryer before putting seeds to germinate.

ROLE OF THE SEED COATS

When Lipscomb and Corley[43] heated cotton seed in a vacuum or in an inert gas like nitrogen at 100° for 26 hours, they obtained a higher percentage of germination in treated than in untreated seed. Other workers as Haberlandt,[26] Wiesner[62] Velten,[60] Honing,[25] Davis,[12] Harrington[20] and others found that certain heat treatments accelerated germination or increased the total percentage of germination or both. In a number of the germination tests conducted by the writer, cotton seed which had been given certain treatments with dry heat appeared to germinate more rapidly than untreated seed. In order to determine whether this increase in rate of germination is due to some wholesome effect of the treatment on the embryo or whether it is due to some favorable change in the seed coat, certain experiments were planned. The results of these tests are set forth in Table V.

In experiment 1, four lots each of 100 seeds of the 1923 crop were used. The seed were carefully selected in order to have the different lots as uniform as possible. Lots 1 and 3 were dried at 60° for 18 hours, then heated at 95° for 12 hours. Lots 2 and 4, which were neither dried nor heated in any way, were used as checks on lots 1 and 3. The seed coats were removed from lots 1 and 2 and the embryos were put to germinate while the seeds of lots 3 and 4 were put to germinate with testas intact. The seeds were germinated between sterile blotters in pans holding 50 seeds each. Since each lot was germinated in two pans the method involved a check on such irregularities of germination that might obtain between one pan and another. Only the combined germination in the two pans representing each lot is given in the table. The number of seeds which had germinated was determined at the end of 25, 45, 57, 76 and 91 hours, and the average length of the radicles (hypocotyl and tap root) was determined at the end of the 72-hour period for the seedlings whose testas had been removed and at the end of the 91-hour period for the seedlings whose testas had been left intact. Only seedlings whose root and hypocotyl were healthy were measured, the number of measured seedlings being given in the table.

When the results of the tests with lots 1 and 2 are compared, it is seen that the embryos of treated seeds germinate more slowly than those of untreated seeds. At the end of 25 hours in the germinator only 62 treated embryos had germinated while 91 of the untreated embryos had begun to grow. At the end of the 45 hour period the germination was 88 and 99 respectively for the two lots and at the close of the 76 hour period the comparison was as 93 to 99. At the end of 76 hours, the radicles of the treated seeds were 26.3 mm. long while those of the untreated seed were 38.3 mm. in length. It is evident both from a comparison of the number of seeds which had germinated at a given time and a consideration of the average length of the hypocotyls that the treatment affected the embryos in such a way as to retard their rate of germination. The difference in the average length of the hypocotyls of the two lots does not appear to be due to more rapid growth of the untreated seedlings for measurements made on seedlings from treated and untreated embryos in lots 1 and 2 of Experiment 2 described below, at the ends of the 72 and 112 hour periods show slightly greater gain in length of the seedling from treated seed.

When a comparison is made of the results of the tests with lots 3 and 4 in which treated and untreated seed were germinated with testas intact, it is apparent that the rate of germination of the treated seed exceeded that of the untreated seed. At the end of the 45-hour period, 23 of the treated compared with 18 of the untreated seeds had germinated, and 12 hours later the relation was at 87 to 65. At the expiration of 91 hours, the germination of lot 3 still exceeded that of lot 4 slightly both in regard to number of seeds germinated and to the average length of the radicles of the seedlings.

In Experiments 2 and 3 the seed used had been treated in the seed-treating machine described in another section of this paper. Here, again, the rate of germination of naked embryos of treated seed was retarded in comparison with that of decorticated untreated seed; and, when seed were put to germinate with testas intact, the treated seed germinated more quickly than the untreated. Experiment 4 was exactly like Experiment 3 except that the sterile blotters were permitted to become slightly dry by standing in the laboratory for a time before the seeds were put to germinate. Under this condition of less rapidly available moisture the number of treated seeds which germinated exceeded the number of untreated so slightly as to be negligible yet the retardation of germination due to the effect of the treatment on the embryos was completely counterbalanced by the increased permeability of the testas of the treated seeds.

The removal of the seed coat permitted much quicker germination of both treated and untreated seed than when seed coats were intact, but the retarded rate of germination of the treated embryos was completely masked and even overcome by the restriction to germination imposed by the intact seed coat of untreated seeds. It is apparent that while the treatment affected the embryo in such a way as to prolong the period required for germination to begin it also produced a profound change in the testa increasing its permeability and more than compensating for the less prompt start of the treated embryos into activity.

The exact nature of this increase in impermeability of the testas of treated seeds has not been determined. It may be that fissures form in the seed coat as the result of heating and thus a more ready access of water to the embryo is provided as suggested by Detmer.[15] Denny[14] found that lipoids, tannins, and pectic substances were factors in determining the permeability of the coats of certain seeds. He found that the permeability of the seed coat of *Arachis hypogea* could be increased 28 to 50 per cent by heating the seeds in dry air at the temperature of boiling water. A much greater increase of permeability occurred when the seed coats were extracted for five minutes in boiling water.

The experiments described above may be summarized in the following words. The effect of desiccating cotton seed at relatively low temperatures varying from 40 to 60° C. for various periods of time has been studied as a means of conditioning the seed to endure without injury, temperatures

of 80 to 95°, which are ordinarily harmful but are necessary to kill the anthracnose fungus. The loss of moisture by the seeds has been determined for the different stages of the treatment. The resistance of the treated seeds increased with increase in temperature and time of desiccation and is correlated with and dependent upon the loss of water during the period of desiccation. The reduction of the water content of seeds to 3.19 per cent of their dry weight fully prepares them to survive the temperature of 95° for 12 hours, such seeds germinating as completely and somewhat more promptly than untreated seeds. The acceleration in the rate of germination resulting from the treatment of properly desiccated seeds at 95° may be attributed to the effect of the treatment on the seed coats. When the preliminary desiccation has not been sufficiently great to fully protect the seeds from the injurious effects of the higher temperatures used in the final period of heating, the seeds show retardation in the rate of growth and more or less marked reduction in the total germination. Less severe injury may manifest itself only as an increase in the time required for germination. This finding agrees with that of Nobbe,[44] Just,[30] Saillard,[47] Haberlandt[29] Höhnel,[34] Dixon,[16, 17] Störmer,[55, 56] Tapke,[57] and Crocker and Groves,[10] who found that temperatures near the maximum increase the time needed for germination.

EFFECT OF STORING DISEASED COTTON SEED OVER DESICCATING CHEMICALS

When it was learned in the early part of these studies with diseased cotton seed that the percentage of seeds carrying viable elements of the anthracnose fungus can be greatly reduced by use of dry heat, the question was raised whether the death of the anthracnose fungus is a result of the excessive loss of water while the seeds are being treated or is due primarily to a direct effect of heat. Is the withdrawal of water during the treatment sufficiently great in itself to produce fatal disruption of vital organization in the fungus protoplasm or is this permanent loss of vitality to be attributed to a direct, perhaps coagulating, effect of the heat on the living substances of the fungus? In order to arrive at an answer to this question, certain experiments were performed to determine the effect of removal of water from the diseased seed by desiccation over chemicals. Diseased seed of the 1919, 1920 and 1921 crops were stored over concentrated H_2SO_4 and dry CaO_2 in desiccators. The lids of the dishes were sealed with petroleum jelly and clamped down to prevent entrance of air from the outside. The dishes were kept at room temperature on a shelf of the general laboratory. Seeds were taken from these dishes from time to time and tested in the usual way for germination and anthracnose. In order to keep record of the degree of desiccation attained by the seeds, a small quantity of seed was sewed in a small cheese-cloth bag and stored in each desiccator. The weight of the bag and of the seed enclosed by it were determined at the beginning of the experiment. Each time seeds were removed from the desiccators for testing, the bag and seeds were again weighed and the amount of water lost by the seeds was recorded.

Table VI gives the data pertaining to these tests. In Experiment 1 seed of the 1919 crop was stored over concentrated H_2SO_4 in a desiccator dish. The seed were placed in the desiccator on July 21, 1920, approximately 9½ months after the seed had been harvested. Lots 1 and 2 were removed 58 and 139 days later respectively and although they had lost 10.2 per cen tand 10.8 per cent of their initial weight as water they still contained a high percentage of seeds with viable anthracnose. Lot 3 was removed from the desiccator after 210 days and at that time 20 per cent of the 50 seeds which were put to germinate or 28 per cent of the 35 seeds which did germinate still contained viable elements of the anthracnose fungus, while only 6 per cent of the seedlings of the check developed anthracnose. At the end of 269 days, when lot 4 was removed, the anthracnose had very largely disappeared from the treated seed, but even then, more than twice as many seedlings in the treated lot became diseased as in the untreated. The seed was by this time 18½ months old and had lost 11 per cent of their initial weight as water. Dry weight determinations made in the usual way on untreated seed gave an average of 10.88 per cent and indicate that 11 per cent represents the total initial water content of the seeds. This is a far more complete desiccation than is brought about by the dry heat treatments effective for freeing seed from anthracnose.

In Experiments 3 and 4, seeds of the 1920 crop were put to desiccate over con. H_2SO_4 and CaO_2. The experiments were begun on July 12, 1921, when the seeds were approximately 9½ months old and the first lot was removed from each desiccator 71 days later. When untreated seed were tested on April 6, 1921, approximately 97 days before they were put to desiccate, seedlings from 52 per cent of the seeds developed anthracnose. By July 30, 1921, 18 days after the experiments were started, the percentage of diseased seedlings in undesiccated seed had dropped to 34, and by August 15, 1921, this percentage had fallen to 10. At the time the first lot was removed from the two desiccators, namely, September 21, 1921, the anthracnose had completely lost its viability in the untreated seeds while it was still alive and capable of growth in 36 per cent and 26 per cent of desiccated seeds in the two experiments. The percentage of anthracnose found in untreated seed on July 30 was maintained till September 21 in desiccated seed, whereas in untreated seed it had completely died out by the latter date. The total water content of the seeds as indicated by a dry-weight determination was 9.6 per cent of their initial weight. The seeds of lot 1, Experiment 3, lost 8.7 per cent of their weight or 90.6 per cent of their total water content during the 71 days they were in the desiccator. Thus it appears from Experiment 3 that the vitality of the anthracnose fungus was prolonged approximately 53 days by removal of the greater part of the water from the seeds.

In Experiment 5, seeds of the 1921 crop were put over con. H_2SO_4 in a desiccator on January 19, 1922, at which time they were 3½ months old. Lot 1 was removed at the end of 196 days and at that time the percentage of diseased seedlings was slightly greater in the treated lot than in the untreated check. When lot 2 was removed, 28 per cent of the 50 seeds put to germinate or 46 per cent of the 35 seeds which did germinate by the end of 17 days in the desiccated lot were found to be carrying living elements of the anthracnose fungus, while at the same time the fungus was still

TABLE

Per Cent of Anthracnose and Germination of Diseased Cotton Seed Stored

Exp. No.	Lot No.	Days in Desiccator	Per Cent Water Lost	Days in Germinator — Per Cent Anthracnose — Treated	Days in Germinator — Per Cent Anthracnose — Check					Days in — Per Cent — Treated
1	1	58	10.2	9 34%	8 34%	2 46%	3 54%	4 62%	5 62%	
	2	139	10.8	39 54%	-------	2 8%	4 50%	----------	8 64%	----------
	3	210	10.8	17 † 20%(= 28%)	17 6%	6 64%	12 68%	17 70%	27 * 70%	----------
	4	269	11.0	14 6% (= 9.6%)	14 4%	9 62%	14 62%	22 60%	41 ‡ 74%	----------
	5	356	11.0	----------	-------	5 64%	6 66%	11 70%	24 76%	70 86%
2	1	60	7.4	27 2%	27 6%	6 64%	12 76%	17 88%	27 * 88%	----------
	2	116	8.3	22 0	22 4%	9 62%	14 64%	22 64%	41 ‡ 70%	----------
	3	225	8.8	----------	-------	6 60%	11 74%	24 78%	70 86%	139‡ 90%
3	1	71	8.7	26 36%	26 0	7 90%	12 92%	26 * 92%		
	2	387	9.4	14 0	14 0	9 86%	14‡ 86%	----------	----------	----------
4	1	71	8.9	26 26%	26 0	7 86%	12 86%	26 * 90%	----------	----------
	2	409	9.6	14 0	14 0	7 90%	14 ‡ 98%	----------	----------	----------
5	1	196	7.8	35 52% (=74%)	35 60%	9 48%	14 52%	19 58%	28 66%	35* 70%
	2	424	------	17 28% (=46%)	17 20%	17 60%	----------	----------	----------	----------
	3	540	7.8	42 16% (= 27%)	42 2%	3 16%	4 30%	10 44%	13 44%	19 46%

*See text for further germination.
†Anthracnose per centage calculated on basis of the number of seeds which were put to germinate and in parenthesis on basis of number which actually germinated.
‡All remaining seeds dead.

VI

FOR VARIOUS PERIODS OVER DESICCATING CHEMICALS. 50 SEEDS IN EACH LOT.

Germinator / Germination						Year When Harvested	Age When Put to Desiccate mo.	Age of Seed When Tested mo.	Desiccating Agent
					Check				
7 62%		9 62%	17 * 66%			1919	9½	11½	Con H_2SO_4
20 72%		31 78%	39 80%	48 * 80%		1919		14	Con H_2SO_4
					6 98%	1919		16½	Con H_2SO_4
					9 96%	1919		18½	Con H_2SO_4
139 ‡ 90%					6 82%	1919		21½	Con H_2SO_4
					6 98%	1919	14½	16½	CaO_2
					9 96%	1919		18½	CaO_2
					6 82%	1919		21½	CaO_2
					7 98%	1920	9½	11½	H_2SO_4
					9 94%	1920		22	H_2SO_4
					7 98%	1920	9½	11½	CaO_2
					7 94%	1920		22½	CaO_2
					9 100%	1921	3½	10	H_2SO_4
					17 94%	1921		17½	H_2SO_4
42 58%	102 * 64%				4 96%	1921		21½	H_2SO_4

viable in only 20 per cent of the seeds of the untreated check. When lot 3 was removed, 16 per cent of the 50 seeds put to germinate or 27 per cent of the 32 seedlings which grew became diseased, while only 2 per cent of the untreated check developed anthracnose. Thus, it appears that in 26 per cent (46%-20%) of the desiccated seeds which germinated in lot 2, the vitality of the anthracnose fungus was prolonged to 17½ months by storage in the desiccator, whereas, under ordinary conditions of laboratory storage, the fungus in these seeds would have perished by this time. Likewise, as indicated by lot 3, the anthracnose fungus was preserved in a viable condition to 21½ months in 25 per cent (27%-2%) of the treated seeds.

The results of Experiment 2 are not in complete harmony with those of the other tests, although the seed were from the same bag as those used in Experiment 1. In tests made at the end of 60 and 116 days fewer seedlings became diseased in treated than in untreated seed. This anomalous outcome is probably dependent on the fact of the more advanced age of the seed when put to desiccate. In Experiment 1, the seed were put to desiccate on July 21, 1920, when they were 9½ months old, while in Experiment 2, they were put in the desiccator on December 18, 1920, at which time they were 14½ months old. When untreated seed were tested on July 1, 1920, 66 per cent of the seedlings became diseased; on Sept. 17, 1920, 34 per cent and on January 18, 1921, just one month after Experiment 2 was begun, only 6 per cent of the seedlings in one lot and none in another became diseased. Thus it is seen that when Experiment 1 was started a large percentage of the seeds were carrying the anthracnose fungus in a viable condition while by the time Experiment 2 was begun the seed had passed through the summer months and the anthracnose fungus in most of the seeds had died as a result of ageing. Until two weeks before Experiment 1 was started, these seed were stored in an out-building; after this time they were kept in the laboratory. During the period of storage in the laboratory before Experiment 2 was begun the water content of the seeds decreased from approximately 11 per cent to approximately 9 per cent. In Experiments 3 and 4, it is shown that the desiccating agents removed all or nearly all the free water from the seeds, and it is assumed that this is likewise the case in Experiments 1 and 2.

From the experiments described above, it is seen that the vitality of the seed-borne elements of the cotton anthracnose fungus is prolonged and not shortened by storing diseased seed at room temperature over desiccating chemicals such as con H_2SO_4 and dry CaO_2. This increase in the length of life of the fungus appears to be conditioned by a pronounced loss of water from the seeds and is probably due primarily to a decided decrease of the already low respiratory activity of the fungus and seed. When diseased seed are treated with dry heat, it is not the desiccation which destroys the life of the anthracnose fungus, but the direct, presumably coagulating effect of the heat on the fungus protoplasm, which appears to be less resistant to heat than the protoplasm of the seed itself. The reduction of the water content of seeds which are being treated is sought only as a means of conditioning the seed to withstand the ordinarily injurious effects of the high temperature necessary to kill the invading fungus.

INDUCED SECONDARY DORMANCY OF COTTONSEEDS

In addition to the increase in length of life of the anthracnose fungus as the result of storing diseased cotton seed in desiccators kept at room temperature, an induced secondary dormancy of more or less prolonged duration was observed to occur in a considerable proportion of the stored seeds. The results of these germination tests are given in part of Table VI.

In Experiment 1, lot 1, 46 per cent of the seeds germinated in 2 days; 54 per cent in 3 days; 62 per cent in 4 days and 62 per cent in 9 days. The seeds were not examined again until the 17th day at which time 66 per cent had germinated. 17 seeds had not germinated by the end of 17 days. Seven of this number were covered with fungus, the seed coats being soft and the embroys dead and partially decayed. Eight of the 17 seeds had seed coats which were extremely dry within and much harder than those of untreated seeds. These seeds had not swollen and had imbibed no water as indicated by the dry condition of the surface of the embryo. When the hard seed coats were removed and the dry embryos were placed on moist blotting paper, 7 of the 8 seeds germinated and produced hypocotyls varying from 1 to 15 mm. within 24 hours, and all germinated in 3 days. The remaining two of the 17 seeds had already imbibed considerable water, the coats being soft and the embryos moist when the seeds were removed from the germinator on the 17th day. These two seeds also germinated promptly when their testas were removed. Obviously, they had already overcome the dormancy imposed by their dry seed coats and were at the point of germination when the seed coats were removed.

In lot 2, which lost 10.8 per cent of its weight while it was in the desiccator, 8 per cent of the seeds germinated in 2 days and 50 per cent in 4 days. Thereafter the percentage of germination increased very slowly until it reached 80 per cent on the 39th day. No further increase had occurred by the 48th day and the seeds were then removed from the test tubes. Six of the 10 which had not germinated were dead and covered with fungous growth while 4 possessed seed coats which were hard and dry within and had apparently absorbed no water. The testas were removed and the seeds were put on moist blotting paper whereupon all germinated and produced radicles about 3 cm. in length within 3 days. In lot 3, germination increased from 64 per cent on the 6th day of 70 per cent on the 17th day while 98 per cent of the seeds of the untreated check germinated in 6 days. By the 27th day, 15 of the desiccated seeds had failed to germinate, 7 having hard and dry testas and 8 being dead. The 7 seeds with hard testas germinated at once when seed coats were removed. In lot 4, the germination increased from 62 per cent on the 9th day to 74 per cent on the 41st day at which time all the remaining ungerminated seeds were dead, while 96 per cent of the untreated check germinated in 9 days. In lot 5, which had been in the desiccator 356 days and had lost 11 per cent of its weight as water, the percentage of germination increased by slow increments from 64 per cent on the 5th day to 90 per cent on the 139th day, by which time all remaining seeds had died. The untreated check gave 82 per cent of germination in 6 days.

In Experiment 2, in which CaO_2 is used as the desiccating agent, germination was prolonged by reason of the induced condition of secondary dormacy. At the end of 27 days, 6 seeds of lot 1 had not germinated, 3 of the 6 being dead and 3 having hard, dry seed coats. The embryos of the 3 seeds with hard coats imbibed water and germinated promptly after the seed coats were removed. In lot 3, germination was greatly prolonged increasing slowly from 60 per cent on the 6th day to 90 per cent on the 139th day at which time all remaining seeds were dead.

In Experiment 3, seed of the 1920 crop were stored over concentrated H_2SO_4 and in Experiment 4 over dry CaO_2. In these two experiments the seeds were highly desiccated as indicated by the percentages of water lost, but germination was only slightly prolonged. In the case of lot 1 of each experiment, the germination was 92 and 90 per cent respectively in 26 days while the checks gave a germination of 98 per cent in 7 days. No note was made of the condition of the seeds which had not yet germinated at the end of 26 days. A portion of these might have germinated later if they had been kept for a longer time. In the case of lot 2 all seeds which had not germinated on the 14th day were dead.

In Experiment 5, seeds of the 1921 crop were stored over concentrated H_2SO_4. The germination of lot 1, removed from the desiccator at the end of 196 days, increased from 48 per cent on the 9th day to 70 per cent on the 35th day. At that time, 8 of the remaining 15 ungerminated seeds were dead and 7 were still alive. The untreated check yielded perfect germination by the 9th day. Only 60 per cent of the seeds of lot 2 germinated in 17 days while 94 per cent of the untreated seeds grew in the same time. Lot 3 was removed from the desiccator at the end of 540 days. The germination increased by small increments from 16 per cent on the 3d day to 64 per cent on the 102d day when the experiment was discontinued. 96 per cent of the untreated seeds germinated in 4 days. The seeds used as checks in all the above mentioned experiments were drawn originally from the same bag as the treated seeds and were handled in the same way except that they were kept in the original bag while the treated seeds were stored in the desiccators.

In 3 out of 5 experiments in which cotton seed were desiccated with chemicals, the period required for complete germination of the viable seeds was markedly prolonged in comparison with the time required for germination of the undesiccated seed. The treated seed are put into a condition of more or less complete secondary dormancy or restricted activity. This is due primarily if not wholly to the desiccation of the testas of such extreme nature that they become non-wetable with water at the germination temperature of about 25° C. In the presence of abundant moisture the desiccated condition of the seed coat is reversed slowly at a rate which differs with individual seeds and which is probably dependent upon the degree of desiccation of individual seeds. Consequently the germination of such seeds is slow and very irregular.

The phenomenon of delayed germination of seeds is exhibited by a very considerable number of species. It is due to conditions which fall into two general categories, namely, those resident in the embryos and those due to restrictions imposed by the seed coats.[7] Thus the embryos of hawthorn

and juniper[6] are dormant and must pass through a period of after-ripening, the time of which may be shortened by appropriate treatments before germination can take place.[8, 13, 19] On the other hand, delayed germination in Xanthium[8, 46] and *Avena fatua*[1] is due not to dormant embryos but primarily to seed coat structures preventing the entrance of oxygen; in *Axyris amaranthodides* and *Chenopodium album*[3] and *Lathyrus maritimus*,[52] to seed coats retarding or preventing the entrance of water; and in Iris,[5] *Alisma plantago*,[11] other water plants,[9] and *Diospyros virginia*,[5] to the great strength of the seed coat which mechanically prevents swelling of the embryo before the latter has imbibed sufficient water to permit germination. With Ilex[7] there obtains a combination of conditions falling in the two general categories mentioned above. The embryos are not dormant but very immature and require a long period of growth before germination occurs but even then the weak hypocotyl is unable to penetrate the woody pericarp until the latter has decayed sufficiently.

Seeds of certain plants which are capable of immediate germination may be thrown into a condition of secondary dormancy. Kidd[10, 11] subjected seeds of *Brassica alba* to high partial pressures of CO_2 and found that the treated seeds became dormant and remained in that condition indefinitely after removal from the inhibiting CO_2 pressures. The dormant condition could be broken only by redrying and rewetting or removal of the testas. Crocker[2] cities other instances in which secondary dormancy has been imposed on seeds by treatments which induced restrictive seed coat changes. However, in the cases of secondary dormancy induced in *Brassica alba* seeds by Kidd and in the cases cited by Crocker the dormant condition was induced while the seeds were in the moist condition favorable for germination. On the contrary, the secondary dormancy exhibited by the cotton seeds described above was induced by excessive drying in desiccators, and is more nearly comparable to the wide-spread hard shell condition characteristic of so many of the Leguminoseae but found in certain other families, among which are the Malvaceae, the family of which cotton is a member. Guppy.[28] The writer has found the hard-shell condition of cotton seeds occurring naturally in only one instance. Some of the seeds used by the writer from the 1924 crop had come to maturity during a season unfavorable for the best development of cotton seeds and among these one seed in a lot of 100 was occasionally found which did not germinate because of its hard shell condition. According to Toole and Drummond,[47] "If the seeds dry out until the moisture content is between five and six per cent, there is a marked development of 'hard seeds' which fail to take up water at all." A similar hardshell condition which retards or prevents germination of beans was described by Gloyer[27] in 1921, who ascribed the condition to extreme drying while stored in artificially heated rooms or when ripening occurs in dry, hot weather.

EFFECT OF STORING INFECTED COTTON SEED IN HYDROGEN AND IN CARBON DIOXIDE

A number of experiments have been made to determine the effect of an atmosphere of hydrogen and of carbon dioxide on the vitality of diseased cotton seed and on the logevity of the spores and mycelium of the anthracnose fungus carried by such seed. As in previous experiments, the seeds used were selected in order to remove all seeds which were, so far as could be judged from external appearances, incapable of germination. These selected seeds were stored in lots of several hundred in 750 cc. bottles provided with 2-holed rubber stoppers. The stoppers were vaselined, pressed tightly into the bottles and tied down with strong cord. The gases used were made to displace the air in the bottles containing the seeds by passing into them by way of 2-holed stoppers quantities of the desired gases equal to several times the volume of the bottles. When the air in the bottles had been displaced, the holes in the stoppers were closed by pushing tightly fitting vaselined glass rods into them. The bottles were were then kept on a laboratory shelf at room temperature. The bottles were recharged with appropriate gases each time samples of seeds were removed for germination tests.

The hydrogen used was produced by the action of zinc and sulphuric acid in a Kipp generator and was passed through wash bottles containing H_2SO_4 KOH solutions. In Experiment 1, the hydrogen was dried by passing it through a tube filled with CaO_2. The carbon dioxide used was produced by the interaction of H_2SO_4 and air slacked lime in a flask provided with suitable outlets and was used without further purification. In Experiments 1 and 2 the untreated seeds used for checks were kept in

TABLE VII

Per Cent of Anthracnose and Germination in Cotton Seeds Stored in Carbon Dioxide and in Hydrogen. 50 Seeds in Each Lot.

Exp. No.	Lot No.	Gas Used	Per Cent Germination		Per Cent Anthracnose		Age of Seed		Year Harvested
			Treated Seed	Untreated Seed	Treated Seed	Untreated Seed	When Stored	When Tested	
1	1	H_2	98	100	6	60	3½ mo.	10 mo.	1921
	2	H_2	90	100	8	32	3½	10½	1921
	3	H_2	88	94	2	20	3½	17½	1921
2	1	CO_2	96	100a	32	60a	3½	10 mo.	1921
	2	CO_2	96	100a	26	?2a	3½	10½	1921
	3	CO_2	92	94a	2	20a	3½	17½	1921
3	1	H_2	94	98	58	90	6 mo.	10½ mo.	1922
	2	H_2	100	96	36	68	6	13	1922
4	1	CO_2	92	96b	50	68b	6	13 mo.	1922
5	1	H_2	92	98	28	28	5 mo.	6 mo.	1923
	2	H_2	98	98	26	26	5 mo.	9 mo.	1923
6	1	CO_2	96	98c	28	28c	5	6 mo.	1923
	2	CO_2	94	98c	40	26c	5	9	1923

aUntreated seeds same as for Exp. 1.
bUntreated seeds same as for Exp. 3.
cUntreated seeds same as for Exp. 5.

an open dish and in Experiments 3 to 6 they were kept in tightly stoppered bottles. The seeds were germinated in test tubes and at a temperature of about 25° C. Each lot tested in each experiment consisted of 50 seeds and the percentage of germination and the percentage of seeds which developed diseased seedlings was determined for each lot at intervals of 6 to 8 days, but only the total percentages obtained at the time the germination test was discarded are given here. The results of these experiments are presented in Table VII.

In two tests as high or higher germination was obtained with the seeds which had been kept in the single gases as with those stored in air. In all remaining tests the treated seeds gave slightly lower germination than the untreated seeds. The differences, however, are slight, and the tests show that cotton seed can suspend aerobic respiration and live in the absence of free oxygen for at least fourteen months.

This situation is not unusual among seeds. Houdas[66] found no reduction in germination of seeds of *Gerbera Jamesoni* after ten years in atmospheres of hydrogen and carbon dioxide. Becquerel[1] and others have shown that seeds may live several months or years in total absence of air, and Laurent[15] and Guillaumin[27] found that seeds of a number of common plants, particularly oleagenous kinds, stored in high vacuum for periods of 5 and 7½ years were conserved better than when stored in air. Babcock[2] found that air dried corn containing 10 per cent of moisture can be stored in CO_2 for a year without harm but under similar storage corn containing 20 per cent of water loses its power to germinate in less than two months. Edquist[5] found that grain can be stored in an atmosphere of pure nitrogen and carbon dioxide without injury to the grain, but the eggs of weevils and other insects are prevented from developing.

In regard to the effect of the treatment on the vitality of the anthracnose fungus, the results of the tests with seed produced in different years are highly discordant. In Experiment 1, in which seed grown in 1921 were stored in hydrogen gas the percentage of anthracnose, computed on the basis of the number of seeds put to germinate, at the end of seven months of storage in the gas was found to be 32 in the untreated and in 8 in the treated seeds and at the end of 14 months of storage the percentage was 20 for the untreated and 2 for the treated seed. In this experiment, a very pronounced reduction but incomplete suppression of disease appears to have resulted from storage of the seeds in hydrogen. In Experiment 2, which differed for Experiment 1 in the single condition that CO_2 was used instead of H_2, 32 per cent of anthracnose was found in untreated and 26 per cent in treated seed after 7 months of storage and 20 per cent was found in the untreated as compared with 2 per cent in the treated after 14 months of storage in the gas. The reductions are much less pronounced than in Experiment 1 except for the longest period of storage in the two gases. In Experiments 3 and 4 some reduction in the amount of disease occurred in the seeds stored in H_2 and CO_2, but the amount is much less than in Experiments 1 and 2. Again, the action of the hydrogen appears to be more efficacious than the CO_2 in reducing disease. In Experiments 5 and 6 in which seed of the 1923 crop were treated, no reduction in viability of the anthracnose had occurred after 4 months storage in either hydrogen or carbon dioxide.

A number of investigators have shown that the permeability of cellular membranes to gases depends upon their state of humidity. Becquerel[1] working with lupin, pea, and bean (Faba) found that their seed coats in an air dry condition are impermeable in all their parts to dry air, but when the same seeds are exposed to an atmosphere saturated with water vapor, the integuments imbibe sufficient moisture to permit the slow passage of gases. No one has studied the permeability of the testas of cotton seeds to hydrogen and carbon dioxide but the work of Becquerel and others indicates that the permeability decreases with increasing desiccation and may actually become nil at the degree of dryness attained when seeds are stored under laboratory conditions. While no moisture determinations were made on the seeds used in the writer's tests described above, it is known that the seeds in Experiments 1 and 2 in which the amount of anthracnose was markedly reduced in the treated lots, were stored in the gases very soon after their arrival at the laboratory. On the other hand, the seeds used in Experiments 3, 4, 5, and 6 in which less reduction of anthracnose occurred, remained for a considerable time in the dry air of the laboratory before the experiments were started and may have become, by reason of more thorough desiccation, less permeable to the gases in which they were kept.

The only conclusion that can safely be drawn from these experiments is that storage in CO_2 and H_2 cannot be relied upon to free cotton seeds from infection by the anthracnose fungus. This agrees with the finding of Barre and Ludwig,[3] who reported that storage of infected cotton in gases did not free the seed from the anthracnose disease.

GENERAL CONCLUSIONS

In order to enable cotton seeds to withstand, without loss of viability, the high temperatures to which they must be subjected in the final stages of the dry heat treatment for anthracnose, it is essential that their water content be reduced to a small fraction of that which is commonly found in seeds stored under ordinary conditions. This preliminary desiccation can be accomplished conveniently and most rapidly by drying the seeds at a temperature too low to harm them. The degree of dehydration required varies in direct manner with the temperature which is to be used in the final stages of the treatment and is fully attained by reducing the water content of seeds which are to be heated for 12 hours at 95° to a value which is equivalent to 3.2 per cent of their air dry weight. Both the temperature and the time of drying are factors of primary significance in this operation. If the temperature is too low, the time of drying can be extended indefinitely without accomplishing the desired degree of desiccation. The absorptive power of the dry air comes into equilibrium with the hydrophyllic properties of the colloids and other constituents of the living cells of the seed before the state of desiccation which will enable the seed to survive high temperatures has been reached. On the other hand, when a temperature which is sufficiently high is used, a more or less definite minimum of time is necessary to produce a degree of desiccation sufficiently pronounced to protect the seeds. A temperature of 45° C. cannot produce

sufficient reduction in the water content of seeds to enable them to endure 95° for 12 hours, but 50° for 36 hours or 60° for 18 hours gives fairly adequate protection. As shown in Part I of these studies on cotton seed treatment, the same kind of time and temperature relation holds for the death of the anthracnose fungus carried on diseased seed. Heating at 45° for 168 hours does not completely free the seed from anthracnose but this result is fully accomplished by 12 hours of heating at 95°.

When seeds have been adequately dehydrated before the application of the high temperature in the second stage of the anthracnose treatment described in Part I of these studies, germination is somewhat more prompt than in untreated seeds. This acceleration of germination of treated seeds is not conditioned by any wholesome effect of the treatment on the embryos but is due entirely to favorable changes in the seed coat which facilitate the passage of water to the embryo; for, when naked embryos of comparable lots of treated and untreated seeds are compared with respect to promptness of germination, the embryos of untreated seeds are seen to germinate more promptly than those of treated seeds. When the seed coats are intact, the less rapid germination of the embryos of treated seeds is completely masked and more than compensated for by the favorable changes which were produced in the seed coats. The increased permeability of the coats of treated seed may be due to the formation of fissures or to the induction of chemical changes in the seed coat by the high temperatures used in the treatment. When the preliminary desiccation has not been sufficiently great to fully protect the seeds from the injurious effects of the higher temperatures used in the final stages of the treatment, the seeds show retardation in rate of growth and more or less marked reduction in total germination. Less severe injury may manifest itself only as an increase in the time required for germination.

Desiccation itself is not responsible for the death of the anthracnose fungus carried on diseased cotton seed; for, when infected seeds are thoroughly dehydrated at room temperature over chemicals, the fungus actually lives longer than in seeds containing a normal amount of water. This increase in length of the life of the fungus appears to be conditioned by the pronounced loss of water from the seeds and is probably due fundamentally to a decided decrease in the already low oxidative and respiratory activities of the fungus and seed. This condition of prolonged viability in the uniformly dry atmosphere of the desiccators is in harmony with the findings of Duvel,[18] who reported that the climate, particularly moisture and temperature of the place where seeds were kept, exerted a marked influence on their length of life, and of Tillotson,[58] who reported that seeds of coniferous trees were little if any affected by geographic location and were better preserved when kept in air-tight glass jars than when stored in bags or other open containers. Moreover, this condition of prolonged viability in the desiccators shows that the death of the anthracnose fungus when diseased seeds are treated with dry heat is not due to desiccation, but is to be attributed to a more direct effect of the heat, which may act by irreversibly coagulating the fungus protoplasm. That the death of the fungus is not due primarily to oxidative changes induced in the fungus protoplasm

is indicated by the experiments of Lipscomb and Corley[43] who reported that the fungus is killed when dry diseased seed are heated at 100° in a vacuum or in an atmosphere of nitrogen. The reduction of the water content of seeds which are to be treated for anthracnose is sought only as a means of conditioning them to withstand the ordinarily injurious effects of the high temperatures necessary to kill the invading fungus.

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